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ADRENALECTOMY AND ITS RELATION TO THE METABOLISM OF THE CAT

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Aub, Forman and Bright (1) in 1922 published an article on effect of adrenalectomy on the total metabolism of the cat. The basal metabolic rate of a series of normal cats was determined by the Benedict method (2). Following these determinations, one adrenal was aseptically removed from each animal. After a suitable period the post-operative metabolic level was measured at intervals for five months. Following single adrenalectomy they found the basal metabolic level to be depressed for a period of 9 to 10 days. This depression was characteristic, since control dummy operations on normal cats did not show so definite a picture. After the initial depression, the oxygen consumption rates of their animals quickly approached the pre-operative level and at the end of the 15th day were equivalent to or above the normal level. The same condition was found to be true five months after the operation.

Previous to the publication of the above results, a small amount of data was obtained by the author from a series of cats in which the experimental conditions above were practically duplicated. As the results obtained differed, in certain aspects, from the figures published by Aub and his coworkers, duplication and further control experiments were carried out and the data are now presented.

METHOD. The method used for the oxygen consumption determinations was the closed system, or internal calorimeter, a description of which, and its special adaptations, is in press. The apparatus resembles that of Benedict (2) in so far as the air circulation, the water absorbers, carbon dioxide absorbers and air dampeners are concerned. Atmospheric conditions were maintained within the animal chamber throughout the periods during which the metabolism was measured. All records were taken at constant temperatures for the individual tests while the metabolic readings

for the entire series were taken at temperatures ranging from 20 to 22.5°C. Before beginning a series of studies, the apparatus was tested for leaks by subjecting it to 50 to 100 cc. mercury pressure.

The basal metabolic rates were determined during the post-absorptive stage, i.e., 16 to 20 hours after feeding. The oxygen consumption rates were measured by taking the period of time necessary for the animals to use 100 cc. of 96 per cent oxygen. This was used as a unit period or record. The time of the unit period ranged from 2.5 to 7 minutes and was affected by the size and metabolic rates of the individual animals. Only those periods in which there were no changes in the temperature of the apparatus and no obvious activity on the part of the animals were recorded in the data presented. Periods during which any animals were obviously asleep were also discarded. The method and the apparatus are adapted to ready selection of the reliable periods and the elimination of periods of questioned variations in the assumed constants. The rule of procedure was to measure ten basal periods and compute the average which was recorded as the basal level.

The cats, selected for gentleness, were kept under animal house conditions for periods of 3 to 10 weeks before the studies began. During the entire period of the experiments the individual maintenance diet consisted of 100 grams raw chopped meat and whole milk ad lib.

The preliminary and normal basal metabolic rates were determined at intervals for each animal over periods of from 2 to 5 weeks after the optimum conditions of nutrition had been reached. As males and females reacted similarly in all experiments and no characteristic differences could be noted, sex has been disregarded in presenting the data.

The operative procedure, under ether anesthesia, was carried out through a single latero-dorsal incision. The entire operation for either single or double adrenalectomy consumed from 30 to 40 minutes. All animals, including controls, were ventilated by means of the air-carbon dioxide mixture of Henderson (3) to remove the excess of ether and were able to stand in 30 minutes or less after closure of the incision.

Single adrenalectomy was performed on a series of 26 cats, including 10 males and 16 females. The series was divided into two equal groups. The left adrenal was removed from the first group and the right adrenal from the second. Following the operations studies were continued for periods ranging from 75 days to 9 months. The oxygen consumption rate and body weights were closely followed, while the respiratory quotient, body temperature and blood sugar concentrations were studied at longer intervals.

Double adrenalectomy was performed on 18 cats. This group included six animals on which the total adrenalectomy was performed at two stages, the second stage following the first by 2 or 3 months. In all 18 of these

experiments, the anal temperature, blood sugar concentration and oxygen consumption rates were determined at two-hour intervals until the animal died. Following operation no food was allowed the animals, but water was given ad lib. The animal room temperature was maintained at 26 to 29°C. in order to control the abnormal loss of body heat.

Two types of control experiments were carried out as checks on the above work. The first type consisted of two adult animals to which ether was administered for one hour. The second type consisted of two dummy operations which duplicated a double adrenalectomy with the

TABLE 1

Graph values expressed as averages for 22 single adrenalectomized cats. Figure 1.

Group A includes those animals whose metabolism, after the initial post-operative drop, returned to normal or above.

Group B includes those animals whose metabolism, after the initial post-operative drop, recovered slightly but not completely.

Group C includes groups A and B or the entire series of 22 cats.

	AVERAGE CALORIES PER KILO BODY WEIGHT PER HOUR			AVERAGE WEIGHT IN KILOGRAMS		
	Group A	Group B	Group C	Group A	Group B	Group C
Number of cats	12	4*	22**	12	4	22
Normal.....	2.72	2.94	2.90	2.77	2.31	2.56
TIME IN MONTHS AFTER SINGLE ADRENALECTOMY						
1 (10)	2.65		2.49	2.69		2.43
1 (20)			2.45			2.46
1 (30)	2.39	2.37	2.23	2.59	2.51	2.68
2	2.41	2.24	2.29	2.80	2.87	2.77
3	2.43		2.41	3.30		2.88
4			2.54			3.37
5	2.77	2.52	2.66	2.80	2.80	2.75
6	3.03	2.36	2.76	2.88	3.20	2.85
7	3.05	2.51	2.88	2.90	3.50	3.06
8	3.30	2.55	2.92	2.75	3.68	3.41

exception that the ligatures were not tied and the adrenals, although exposed and handled, were not excised. In both of these control experiments, the blood sugar level, anal temperature and oxygen consumption rates were determined at regular intervals for 120 to 144 hours after the treatment.

RESULTS. *Single adrenalectomy.* After a careful survey of the mass of data obtained from this series, two apparently different reactions to the operation were noted. The first group or type A, table 1, was that in which the metabolism recovery was complete. The second type B, table 1, was that in which the metabolism recovery was insufficient to reach the pre-operative level.

Group A, figure 1, resembles grossly the results obtained by Aub, Forman and Bright (1). The average metabolism of this group of 12 animals when computed on the basis of calories per kilogram body weight per hour following single adrenalectomy, showed a depression which continued for approximately 30 days, at which time the metabolism rate begins a recovery toward the pre-operative normal level, and by the fifth month the recovery

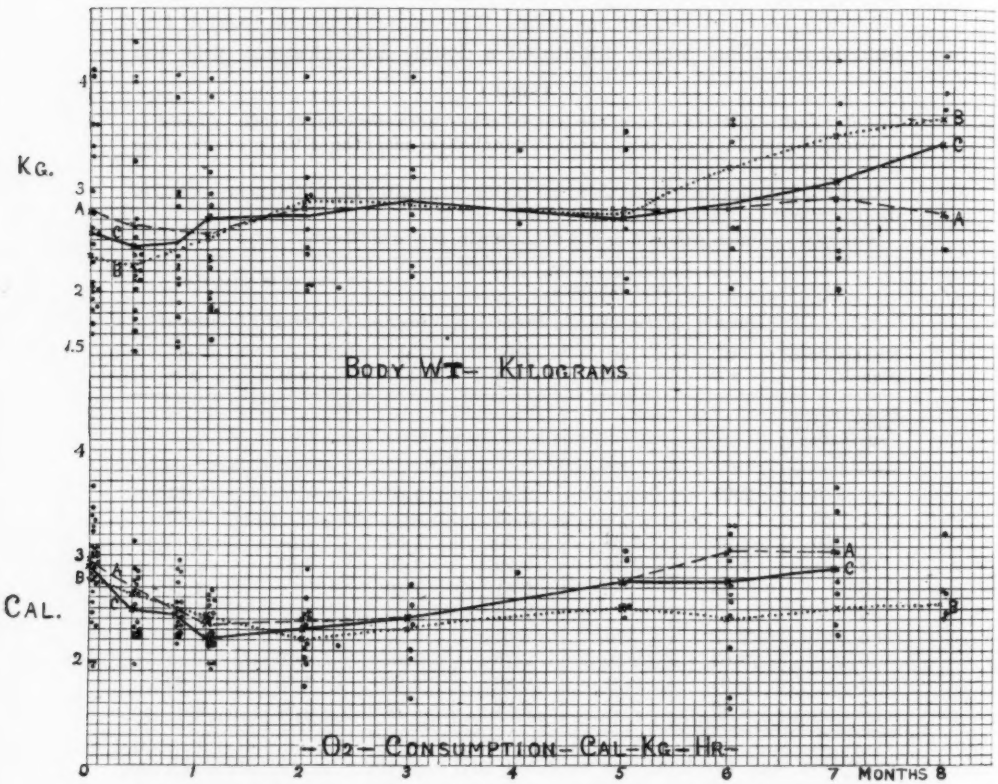

Fig. 1. Plotted values of table 1. Observations on 22 single adrenalectomized cats

was complete. The post-operative basal level after the fifth month was maintained at or slightly above the pre-operative normal level until the end of the experiment.

The body weight of group A showed a corresponding initial drop following the operation for a period of 30 days, after which there was a quick recovery to the original level where it was maintained, with slight variations, until the experiments were concluded. An example of this group is cat 30, table 2.

Group B, including five animals, shows a value very similar to that of A as to the average rate of metabolism for the first 30 days. The post-operative depression, however, continued until the second month at which time the rate approached the pre-operative basal level. By the fifth month a new basal level 14 per cent below the original pre-operative level was established. This new level was maintained until the end of the experiments.

The average body weight value of group B, on the other hand, differed markedly from corresponding values of group A. The weight was depressed for twelve days following the operation, after which the normal

TABLE 2

Typical case—Group A

Female cat 30

DATE	WEIGHT	BASAL METABOLIC RATE PER KILOGRAM HOUR	REMARKS
1923	*kgm.*	*calories*	
September 20 to October 20	2.55	3.04	Adult, well-nourished
October 25	Right adrenalectomy		
			Ether anesthesia
November 3	2.37	2.78	Healed, normal
November 15	2.36	2.54	
November 22	2.44	2.53	
December 7	2.41	2.60	
1924			
January 3	2.60	2.84	
May 10	2.60	2.89	
May 11	2.65	3.06	
May 13	2.60	3.67	Restless
June 12	2.45	2.98	
June 16	2.35	3.43	Restless
July 8	2.48	3.30	

was quickly reached and, after reaching normal, continued to increase until the second month when a new level was maintained until the fifth month. From the fifth month on the values gradually increased until the experiment terminated. Cat 29 is typical of this series, table 3. The only physical explanation of this difference between groups A and B, lies in the great increase in body weight or adipose tissue of the animals of group A.

Group C, table 1, represents the mean average of groups A and B, or the average readings for the entire number of single adrenalectomized animals. Generally speaking, the average metabolism curve, figure 1, very closely approaches the normal pre-operative level at the fifth month

and at the seventh month the values are definitely normal or slightly above that level. The body weight curve 30 days after single adrenalectomy is definitely above normal. The values are maintained at slightly above this level until the fifth month when an increase was noted until the end of the experiment.

The explanation of the difference between the two groups of this series, A and B, seems to rest on the excess body weight of the animals included in group B. Every animal was an adult and from a post mortem examination the excess weight was due to a general deposit of fat. This excess fat deposit was the only variant factor between groups A and B, and very

TABLE 3

Typical case—Group B

Male cat 29

DATE	WEIGHT	BASAL METABOLIC RATE PER KILOGRAM HOUR	REMARKS
1923	*kgm.*	*calories*	
September 18 to October 20	3.40	2.82	Adult, well-nourished
October 25	Right adrenalectomy		Ether anesthesia
November 10	3.05	2.33	Almost healed
November 30	3.40	2.17	Normal
1924			
January 2	3.66	2.02	
April 30	3.86	1.66	Asleep (?)
May 9	4.00	2.22	Very fat
May 30	4.20	2.35	
June 10	4.10	2.24	
June 12	4.15	2.40	
July 8	4.20	2.42	

possibly was the one which caused the difference between the respective oxygen consumption rates of the two groups.

The next possibility which might be considered as a causation factor would be that of actual adrenal insufficiency. From histological examinations of the respective adrenals of groups A and B, no differences were noticeable. And a comparison of the increased weights accompanying the definite hypertrophy of the second adrenals of group B, with those of group A, showed a negligible difference.

Assuming that the metabolism of adipose tissue is very low, the explanation of the divergence between the oxygen consumption values of groups A and B would seem to rest on the body weight differences rather than on an actual adrenal insufficiency. Rolly (4) found, on comparing the reactions of obese patients with normal individuals, following a test meal,

that the metabolic values of the obese were definitely subnormal. He concludes that the basal metabolism of obese subjects when computed on the body weight basis seems to indicate the presence of a large mass of approximately inactive tissue. Excess body fat was indicated.

As a further indication of this assumption, when the metabolic rate is corrected for the excess body weight, i.e., using the original normal body weight, the values of group B approximate or are slightly above the original normal level, even before the fifth month. In other words, the

TABLE 4

Average value of graphs—figure 2

Data from 18 cats following double adrenalectomy

	NUMBER OF ANIMALS	CALORIES PER KILOGKAM HOUR	ANAL TEMPERATURE	BLOOD SUGAR	SURVIVAL
Normal.....	18	2.98	38.50	0.102	
HOURS AFTER DOUBLE ADRENALECTOMY			°C.	per cent	
1	9	2.25	36.20	0.137	Shortest 7½ hours
2	11	2.53	36.94	0.162	Longest 104 hours
3	10	2.62	37.50	0.120	
4	4	2.43	38.20	0.100	*Average 40.1 hours
5	10	2.23	38.40	0.108	
6	5	2.74	38.50	0.098	
8	6	2.39	39.20	0.110	
9	8	2.30	39.10	0.108	
12	9	2.32	39.20	0.096	
16	3	2.16	39.04	0.090	
18	10	1.95	38.75	0.087	
24	9	1.92	38.60	0.077	
30	8	1.85	38.20	0.060	
36	7	1.50	36.1-35.2	0.054	

*Animals whose temperatures had not recovered at the fourth hour are not included in averages.

total metabolism of group B was equivalent to or above the normal basal level.

The changes in respiratory quotient, body temperature and blood sugar concentration, following single adrenalectomy, with the exception of the first four days after the operation, are negligible and at the termination of the experiments, the variations were within the limit of error.

Double adrenalectomy. The average oxygen consumption rate, table 4, following this operation drops quickly within the first hour, and then gradually recovers toward normal until the sixth hour. Following this period the basal metabolic rate shows a very gradual depression which continues

An individual experiment typical of the reactions of a double adrenalectomized animal is shown, cat 34, table 5.

In table 6 two sets of experiments have been recorded as controls for the single and double adrenalectomy experiments. By comparing the data from the ether controls the initial hyperglycemia may be explained. The effect of the anesthesia on the body temperature and oxygen consump-

TABLE 6

Control Experiments

SEX	WEIGHT	TREATMENT	HOURS AFTER TREATMENT	O_2 CONSUMPTION PER KILOGRAM HOUR	ANAL TEMPERATURE	BLOOD SUGAR
	kgm.			*calories*	*°C.*	*per cent*
Male	3.1	Ether 1 hour	Normal	2.84	38.4	0.097
			2	2.65	38.3	0.096
			6	2.95	37.7	0.105
			24	2.88	37.9	0.092
			144	2.93	38.4	0.090
Female	3.0	Ether 1 hour	Normal	3.01	38.1	0.087
			2	2.76	37.8	0.100
			6	2.85	38.1	0.120
			24	3.25	38.3	0.110
			144	2.95	39.1	0.110
Female	2.8	Dummy operation	Normal	2.97	38.3	0.085
			2	2.55	37.6	0.210
			4	2.85	38.1	0.098
			18	2.81	38.5	0.095
			120	3.25	38.5	0.103
Female	2.6	Dummy operation	Normal	2.78	39.4	0.090
			2	2.46	39.0	0.280
			4	2.98	39.1	0.290
			18	2.94	39.0	0.190
			120	2.92	39.3	0.096

Both animals subjected to dummy operation were completely healed in five days. No infection in either case.

tion, however, was very slight except during the first two or three hours. The dummy operations further explain the initial hyperglycemia although the effect on the body temperature was negligible. Oxygen consumption was lowered approximately the same as in similar experiments reported by Aub (1).

Following double adrenalectomy in cats the critical turning point or beginning of collapse seems to precede death from four to eight hours. This condition is easily noted by the rapid drop in body temperature, a

weakened physical condition and obviously low blood pressure. Blood samples taken even shortly preceding this stage are obtained with great difficulty. Respiratory difficulty was usual and frequently a reversed respiratory pause was noted. Certain of these conditions duplicate Elliot's (5) findings which show that blood pressure falls when a condition of collapse develops; also data by Killian (6) and Hovens (7) who state that low blood pressure in Addison's disease is accompanied by low blood sugar. Strehl and Weiss (8), working with cats, state that body temperature following adrenalectomy quickly became subnormal and then gradually decreased until the animals died, while blood pressure dropped 20 to 30 mm. mercury pressure immediately and then gradually fell until death.

The periods of survival of the 18 double adrenalectomized animals ranged from 7½ to 104 hours respectively, while the average for the entire group was 40.1 hours. This average survival period is considerably longer than that reported by Gradinescu (9) whose longest survivals were 36 and 53 hours, or those of Moore and Purinton (10) who note that all of their animals died within 24 hours following double adrenalectomy.

One observation was made which might be of interest in relation to the survival period. The shortest periods were invariably those of animals which on autopsy proved to be quite fat and generally the length of the survival period varied inversely to the value of the excess body fat.

SUMMARY

1. Experiments are reported tending to show the effect of single and double adrenalectomy upon body weight, blood sugar, anal temperature and oxygen consumption.

2. The total metabolism of the cat following single adrenalectomy is initially depressed but gradually returns to or above the pre-operative level.

3. The metabolic rate following single adrenalectomy when computed on a per kilogram body weight basis shows an initial depression and a gradual recovery toward normal. In 25 per cent of the cases studied over a period of 9 months a new basal level, 14 per cent below their respective normals, was established at the fifth month and was maintained until the end of the experiments. In 75 per cent of the cases studied the metabolic rate had returned to or slightly above the pre-operative normal at the fifth month.

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(2) Benedict and Homans: Journ. Med. Res., N. S., 1912, xx, 409.
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and at the seventh month the values are definitely normal or slightly above that level. The body weight curve 30 days after single adrenalectomy is definitely above normal. The values are maintained at slightly above this level until the fifth month when an increase was noted until the end of the experiment.

The explanation of the difference between the two groups of this series, A and B, seems to rest on the excess body weight of the animals included in group B. Every animal was an adult and from a post mortem examination the excess weight was due to a general deposit of fat. This excess fat deposit was the only variant factor between groups A and B, and very

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2	11	2.53	36.94	0.162	Longest 104 hours
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4	4	2.43	38.20	0.100	*Average 40.1 hours
5	10	2.23	38.40	0.108	
6	5	2.74	38.50	0.098	
8	6	2.39	39.20	0.110	
9	8	2.30	39.10	0.108	
12	9	2.32	39.20	0.096	
16	3	2.16	39.04	0.090	
18	10	1.95	38.75	0.087	
24	9	1.92	38.60	0.077	
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*Animals whose temperatures had not recovered at the fourth hour are not included in averages.

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Double adrenalectomy. The average oxygen consumption rate, table 4, following this operation drops quickly within the first hour, and then gradually recovers toward normal until the sixth hour. Following this period the basal metabolic rate shows a very gradual depression which continues

until death. The metabolic level during the period of collapse, irrespective of the rapid, shallow respirations, was quite low and readings taken

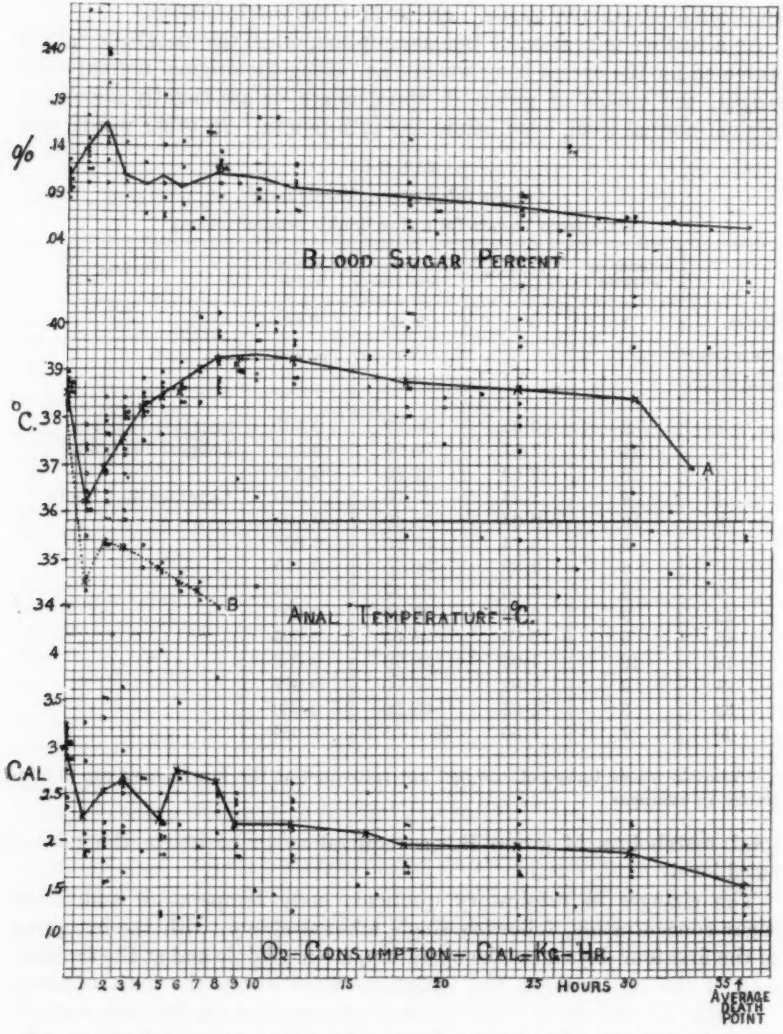

Fig. 2. Plotted values of table 2. Observations on 18 double adrenalectomized cats.

10 to 30 minutes preceding death even though respiration was regular varied from 50 to 60 per cent below the original normal.

The average anal temperatures of the series show a very rapid depression during the first hour. The temperature then gradually rises to normal by the fifth hour and at the tenth hour reaches a peak from which there is a gradual decline until the normal level is reached at the thirteenth hour.

The body temperature of the group of animals studied, varied but little from the normal limits until the beginning of collapse. The body temperature, from 4 to 6 hours before the death, gradually but rapidly fell until at death it was 2.5 to 4.5C°. below the original normal.

TABLE 5

A representative experiment following double adrenalectomy

Male cat 34. Weight 3.1 kgm.

	TEMPERATURE		OXYGEN CONSUMPTION		BLOOD SUGAR	REMARKS
	Room	Anal	Per kilogram minute	Per kilogram hour		
Normal	27	38.8	10.42	3.02	0.102	
HOURS AFTER DOUBLE ADRENALECTOMY	*°C.*	*°C.*	*cc.*	*calories*	*per cent*	
2	28	36.4	6.63	1.92	0.172	Walks steadily
5	29	37.6	8.37	2.42	0.085	Resists strongly
11	27	38.8	6.28	1.82	0.072	
18	26	36.3	5.96	1.73	0.083	
24	28	37.3	6.62	1.90	0.072	Sleeps
29	28	36.9	5.70	1.65		Sleeps
31	29	35.3	4.38	1.27	0.062	Blood pressure very low
36	29	35.5			0.054	
41	27	34.3				Dead

Normal 24 hour sample of urine:
Volume 54 cc.
Creatinine 162 mgm. per 100 cc.
Reducing power 0.15 per cent (picric acid)
36 hours after operation:
Volume 26 cc.
Creatinine 397 mgm. per 100 cc.
Reducing power 0.60 per cent.
38 hours after operation:
Volume 8 cc.
Creatinine 402 mgm. per 100 cc.
Reducing power 0.57 per cent.

The blood sugar concentrations during the two hours after operation showed a hyperglycemia which gradually decreased to normal by the fifth hour. The sugar percentage varied irregularly but was generally higher than normal until the tenth hour after which it was progressively subnormal until death.

An individual experiment typical of the reactions of a double adrenalectomized animal is shown, cat 34, table 5.

In table 6 two sets of experiments have been recorded as controls for the single and double adrenalectomy experiments. By comparing the data from the ether controls the initial hyperglycemia may be explained. The effect of the anesthesia on the body temperature and oxygen consump-

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	kgm.			*calories*	*°C.*	*per cent*
Male	3.1	Ether 1 hour	Normal	2.84	38.4	0.097
			2	2.65	38.3	0.096
			6	2.95	37.7	0.105
			24	2.88	37.9	0.092
			144	2.93	38.4	0.090
Female	3.0	Ether 1 hour	Normal	3.01	38.1	0.087
			2	2.76	37.8	0.100
			6	2.85	38.1	0.120
			24	3.25	38.3	0.110
			144	2.95	39.1	0.110
Female	2.8	Dummy operation	Normal	2.97	38.3	0.085
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The periods of survival of the 18 double adrenalectomized animals ranged from $7\frac{1}{2}$ to 104 hours respectively, while the average for the entire group was 40.1 hours. This average survival period is considerably longer than that reported by Gradinescu (9) whose longest survivals were 36 and 53 hours, or those of Moore and Purinton (10) who note that all of their animals died within 24 hours following double adrenalectomy.

One observation was made which might be of interest in relation to the survival period. The shortest periods were invariably those of animals which on autopsy proved to be quite fat and generally the length of the survival period varied inversely to the value of the excess body fat.

SUMMARY

1. Experiments are reported tending to show the effect of single and double adrenalectomy upon body weight, blood sugar, anal temperature and oxygen consumption.

2. The total metabolism of the cat following single adrenalectomy is initially depressed but gradually returns to or above the pre-operative level.

3. The metabolic rate following single adrenalectomy when computed on a per kilogram body weight basis shows an initial depression and a gradual recovery toward normal. In 25 per cent of the cases studied over a period of 9 months a new basal level, 14 per cent below their respective normals, was established at the fifth month and was maintained until the end of the experiments. In 75 per cent of the cases studied the metabolic rate had returned to or slightly above the pre-operative normal at the fifth month.

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ERRATUM

The paper by Marshall and Crane in the November number of the last volume (1924, LXX, 465) contains an error.

On page 483, tenth line from the bottom, substitute the word "greater" for the word "lesser." This line, corrected, should read:

"any body must be less in the case of the period of greater urine flow. We do."

THE SECRETORY FUNCTION OF THE RENAL TUBULES

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It is now generally recognized that the first process in the formation of urine by the kidney consists in the filtration of the non-colloid constituents of the plasma through the glomeruli. This necessitates the reabsorption normally of glucose and at times of chloride during the passage of fluid along the tubule. That this occurs in the frog's kidney has been recently experimentally demonstrated by Wearn (1). Is the concentration of substances such as urea, phosphate or sulphate brought about entirely by the reabsorption of water, by secretion of these bodies by certain parts of the tubule, or by a combination of both processes? Does the kidney *secrete* at all? What is the rate of glomerular filtration under any given set of conditions? To what extent does the kidney eliminate substances which are not preformed in the blood (e.g., ammonia, hippuric acid)? These questions which are more or less intimately connected must be decided before much further advance in our knowledge of the mechanism of renal excretion can be made, and before the more difficult task of the localization of function in the different parts of the tubule can be attacked with much hope of success.

Marshall and Vickers (2) have recently published evidence that the mammalian kidney can *secrete* phenol red (phenolsulphonephthalein). References to the older literature and evidence on the question of tubular secretion can be found in their article. Their investigations showed *1*, that an accumulation of phenolsulphonephthalein in the cortex of the non-secreting kidney occurs after intravenous injection; *2* that this substance is present in the blood to a great extent in some non-diffusible combination with the colloids, and *3*, that under certain conditions after injection the substance in its free form is not present in a sufficient concentration in the arterial blood to account for the quantity eliminated in the urine even if filtration of all the plasma is assumed to occur. The only conclusion possible seemed that the dye was secreted by the tubules after a temporary storage in the renal cells. Results obtained independently by de Haan (3) at the same time on the relative concentration of certain dyes and urea by the rabbit's kidney support the idea of the secretion of certain dyes, although it is fair to state that de Haan interprets his results without

invoking secretion. In a paper published shortly after the above (4), we concluded from a study of the effect of temporary anemia on the elimination of different urinary constituents and from an analogy to the secretion of phthalein that urea, sulphate, and phosphate were probably secreted. However, it was clearly appreciated that the evidence was indirect and that a possible, but what seemed at the time unlikely, explanation of the results could be given in terms of filtration and reabsorption alone.

Mayrs (5), in view of the evidence for the secretion of certain dyes by the kidney, has quite recently examined the rabbit's kidney for storage of urea and sulphate after the injection of a mixture of these substances with negative results, and concluded that they are eliminated entirely by filtration. In a somewhat later paper, Mayrs (6) presents evidence for the secretion of uric acid by the fowl's kidney and believes that the secretion of dyes in the mammal is a survival of a process occurring in a more primitive kidney. White (7) on the basis of studies of the plasma and urine concentrations as well as rates of elimination of urea, sulphate, phosphate and glucose believes that secretion of these substances by the tubule is necessary to explain his results. Underhill (8) has carried out somewhat similar studies on urea, uric acid, creatinine, and phosphate in the cat, and has come to about the same conclusion. On the other hand, Bieter and Hirschfelder (9) have published results which they believe definitely prove that in the frog, dyes (phenolsulphonephthalein and indigo carmine) are eliminated entirely by filtration and that storage or secretion by the renal cells does not occur.

The above brief summary of quite recent work indicates the interest of many workers in the question of tubular secretion, but also reveals a conflict of opinion. In this paper, we shall give the results of investigations continuing and attempting to extend the work of Marshall and Vickers. We believe our results on the excretion of urea and phenol red by the frog and mammal clear up much of the uncertainty regarding secretion by suggesting two criteria by which the question of the secretion of any substance can be examined. A more detailed consideration of the results of the various workers mentioned above in the light of our data would appear to reconcile many apparent contradictions. It is intended to deal only with the question of the secretion of urea and phenolsulphonephthalein in this paper.

Methods. Large bull frogs (*Rana catesbiana*) varying from about 500 to 900 grams in weight were used in all the experiments on the frog's kidney. The frogs were kept in a tank with running water at a temperature between 13 and 18°C. They were brought to the laboratory before the experiments and confined in individual cages placed in a pan of water. About one half of the animals used in these experiments were allowed to

remain at laboratory temperature over night before commencing any experiment, the others being taken directly from the frog room. The laboratory temperature varied from about 17 to 22°C. The animals were not fed during the experiments. A fresh supply of frogs was obtained about every six weeks.

Unless otherwise stated no anesthetic was administered to the frogs. Urine was obtained by catheter from the bladder and was in most cases uncontaminated with fecal material. Blood was drawn by means of a syringe and needle inserted through the skin into the ventricle. Powdered potassium oxalate was used to prevent coagulation. Intravenous injections were made by exposing a small skin vein by an incision on the dorsal surface. Diuresis was induced by the introduction of distilled water into a lymph sac or by the intravenous injection of Ringer's solution. Anesthesia was induced, when desired, by means of either ether vapor or by the injection of urethane (0.25 gram per 100 grams) into a lymph sac. The ureters were exposed by two longitudinal incisions on the dorsal surface, and cannulated with small bent glass tubes. Dogs or rabbits were used for the necessary experiments on the mammalian kidney. In all experiments they were anesthetized with paraldehyde and urethane respectively. Urine was collected from cannulae in the ureters. Blood was drawn from the carotid artery, and injections made intravenously into a leg vein.

Urea was estimated by means of urease, aeration, and titration. Pyrex test tubes of 200 × 20 mm., 0.01 N acid and 0.02 N alkali, and carefully calibrated micro-burettes were used in the determination. Methyl red served as indicator. Since very small quantities of material were frequently used, great care in the estimations was necessary. Blank determinations were frequently made on the apparatus and reagents. They never amounted to more than 0.01 cc. of 0.02 N acid, while control determinations with pure urea solutions indicated that the procedure was sufficiently accurate for the present purpose. Tissues were finely ground and suspended in a suitable phosphate buffer mixture for analysis. Ammonia determinations were made by aeration. Phenolsulphonephthalein was estimated colorimetrically. Determinations on plasma were made either by direct comparison with a standard containing the same amount of normal plasma, or by the alcohol method of Marshall and Vickers (2). Whole blood and tissue estimations were always made by the alcohol procedure. Bicarbonate was estimated in plasma and urine by means of the Van Slyke-Stadie apparatus (10).

Concentration of substances by the kidney. A systematic study of the comparative physiology of the kidney has been planned and begun in this laboratory. Observations on the frog's kidney indicate that urea is concentrated to a much greater extent than many other bodies normally present in the blood or urine of this animal. The relative concentrations

of urea, uric acid, glucose, phosphate, chloride and bicarbonate have been determined. Phosphate, chloride, bicarbonate, and glucose[1] occurred in the urine normally only in traces or small amounts in our frogs. Phosphate may be more concentrated in the urine than in the blood, either after drying the animal or injecting phosphate, while under other conditions it is usually less concentrated. A concentration of more than four times has not been observed for phosphate, and in these experiments a simultaneous determination of the concentration ratio for urea has shown the latter to be much greater. Thus in one experiment in which the frog was dried, urea was concentrated 65 and phosphate 4.5 times. Glucose (after phloridzin or injection of glucose) may be more concentrated in urine than in blood; but, as in the case of phosphate, urea always has a much higher concentration ratio than the sugar. Neither chloride nor bicarbonate have been found to occur in greater concentration in the urine than in the plasma, even if introduced into the frog until toxic symptoms appear. Phenol red may be concentrated to about the same extent or somewhat better than urea. Przylecki's (11) observation that the molecular concentration of the urine is never greater than that of the plasma is confirmed. The details and experimental data relating to the above statements will be more fully discussed in a separate communication by one of us (M. M. C.) on the function of the kidney in the frog.

Data which in a general way are similar to the above have been recently collected by Mayrs (6) in a study of the bird's kidney, with the difference, however, that uric acid was the substance having a much higher concentration ratio than other bodies. Mayrs has interpreted this to mean that uric acid is secreted by the fowl's kidney. The same interpretation would appear to be suggested in the case of urea in the frog, but, of course, one can assume in either case a very great reabsorption of all other bodies than uric acid or urea.

In the case of the mammal,[2] it is known that glucose, chloride, bicarbonate and phosphate may be less concentrated in the urine than in the plasma; so that if the glomerular filtrate contains these substances in approximately the same concentrations as they exist in the blood, they must be reabsorbed by the tubule. Marshall (12) found creatinine normally concentrated more than urea in the dog, but the present uncertainty of the estimation or indeed existence (13) of creatinine in blood prevents any conclusion being drawn. Mayrs (14) found that after injection of a mixture of two

[1] We have for convenience designated as glucose the total reducing substances in blood and urine. Where the term is used it must be understood with this reservation.

[2] In discussing the mammalian kidney, we refer to data obtained on the dog, rabbit and man, but do not infer that all the statements have been established for the three species. It is quite possible that different species of mammals may differ somewhat in the details of their renal activity.

substances into the rabbit sulphate, phosphate and creatinine have about the same concentration ratios, while urea has a much lower one. Underhill's (8) results in the cat show that after raising the plasma concentrations by injection, creatinine may be concentrated from two to five times more than urea, and phosphate to about the same extent as urea or up to nearly three times more. In two experiments, uric acid appeared to be concentrated as well as creatinine. White (7) finds in the dog that phosphate is much better concentrated than urea, and slightly better than sulphate, when the plasma concentrations are increased by injection; but if the phosphate concentration in the blood is normal and sulphate is injected, sulphate is much better concentrated than phosphate. Urea, also, would certainly have a higher concentration ratio than phosphate under these latter conditions, but when phosphate is injected its ratio is

TABLE 1

WEIGHT	INJECTION	URINE PER MINUTE	UREA (MILLIGRAM PER CENT)		CONCENTRATION	CO_2 (VOLUME PER CENT)		CONCENTRATION
			Plasma	Urine		Plasma	Urine	
		cc.						
2960	8% $NaHCO_3$ / 6% Urea, 40 cc.	3.7	214	400	1.87	107	204	1.91
2700	8% $NaHCO_3$ / 6% Urea, 50 cc.	5.6	234	444	1.93	134	231	1.72
1800	8% $NaHCO_3$ 30 cc.		25	54	2.16	117	209	1.80
1950	8% $NaHCO_3$ 28 cc.	2.0	30	53	1.77	115	191	1.66
2350	8% $NaHCO_3$ 40 cc.	1.3	33	87	2.63	111	237	2.13

higher than that of urea. A necessary corollary of these results is that if urea is secreted, so is phosphate when injected, but not always when the plasma level is normal.[3]

A few experiments have been carried out on the relative concentration ratios of urea and bicarbonate in the rabbit. The general method was similar to that used by Mayrs (14). Table 1 gives a summary of the results. The bicarbonate results are really the total carbon dioxide, and hence the concentration ratios for bicarbonate are somewhat too low.

The concentration ratios for urea and bicarbonate are seen to be about the same in two, and nearly the same in the other three experiments. This means that bicarbonate is secreted, if urea is, in these experiments. The secretion of bicarbonate necessitates the assumption that it is secreted at one time and not at another; or that, under certain conditions all of the

[3] Of course, it is also possible to interpret the phosphate results by assuming that phosphate is secreted at all times, but sometimes all of the secreted phosphate as well as a part of that in the glomerular filtrate is reabsorbed.

secreted bicarbonate is reabsorbed along with a portion of that occurring in the glomerular filtrate. This is obvious from the fact that in acid urines bicarbonate is present only in traces and in a much lower concentration than that of the plasma (15). The same line of reasoning can be applied to glucose and chloride for the mammal. This means that if urea is secreted, practically all urinary constituents are actively secreted as well as filtered. The determination of concentration ratios for the mammal would appear to give no definite indication (as it does in the bird and frog) for the secretion of any of the normal urinary constituents examined, but would rather tend to suggest the opposite conclusion. As Mayrs (5) has pointed out, certain dyes have a much higher concentration ratio than any of the normal urinary constituents for which the ratio has been determined. This is in line with our former results on phenolsulphonephthalein. However, there are many inconsistencies in our knowledge of concentration ratios, and we shall return to this question later in the present paper.

TABLE 2

Frog 44

	UREA	AMMONIA (AS UREA)	UREA + AMMONIA
Blood	11.1		11.1
Heart	7.4	7.6	15.0
Lung	5.7	3.0	8.7
Stomach	5.3	4.5	9.8
Leg muscle	8.3	5.7	14.0
Liver	25.4	4.6	30.0
Bile			8.2
Kidney	**47.1**	7.9	**55.0**
Urine	**20.5**	4.5	**25.0**

Concentration of urea and phenol red in the cells of the frog's kidney. The process of secretion of phenol red by the dog's kidney as outlined by Marshall and Vickers (2) involves the storage or concentration of the dye in the renal cells. The experiments summarized below indicate that both urea and phenol red occur in the frog's renal cells in a much higher concentration than in the blood or other tissues.

Diuresis was induced to minimize the error due to the urine contained in the kidney. Urine was collected for a period which was just about long enough to give a sufficient quantity for analysis, the frog stunned, if unanesthetized, and the kidneys immediately removed. A sample of blood was then taken. Urea is present in all body tissues and fluids (except fat and the urinary tract) in approximately the same concentration as in the blood in the dog (16). This has been confirmed for the guinea pig (17), rabbit (17), chicken (17) and turtle (17), and it is, therefore, probably true for

the frog. The lack of data on the frog, however, led us to include in two or three experiments analyses of other tissues than kidney. Table 2 gives a summary of one of these experiments. Due to the fact that Gad-Anderson (18) claims that frog's muscle readily changes urea to ammonia, we have expressed the results as urea, and as urea plus ammonia calculated as urea. It makes no essential difference in our conclusions in regard to the kidney which figures are taken. All figures represent concentrations in milligrams per cent.

The outstanding fact about these figures is that the kidney contains urea in over twice the concentration that it is present in the urine, and about five times that in the blood. Regardless of how much urine or blood one assumes to be present in the organ, the conclusion is inevitable that urea is concentrated in the renal cells. The high concentration of urea in the liver is noticeable and may mean that the liver of the frog secretes urea, as has been claimed for the liver of the dog fish (19).

TABLE 3

DATE	EXPERIMENT NUMBER	MILLIGRAM PER CENT OF UREA IN		
		Blood	Urine	Kidney
4-9-24	Frog 35B*	24	72	107
4-8-24	Frog 36*		137†	104
4-5-24	Frog 35*	10	16	46
4-7-24	Frog 29	7	28	55
4-22-24	Frog 43	10	13	23

* Figures represent urea + ammonia calculated as urea.
† No diuretic.

Table 3 gives the data from other experiments in regard to the urea content of the kidney, urine and blood. Nos. 29, 35B and 43 were done under urethane, nos. 35 and 36 without anesthesia. In frogs 35B and 36, the kidney was divided into dorsal and ventral portions. The dorsal part of 35B (0.61 gram) contained 137, and the ventral (1.15 grams) 90 mgm. per cent of urea. In frog 36 the figures were: dorsal (0.76 gram) 117; ventral (0.65 gram), 107 mgm. per cent. We shall return to a discussion of the meaning of this difference later.

With the exception of frog 36, the kidney is seen to contain urea in much higher concentration than either the urine or blood, which we can only interpret as indicating a storage in the renal cells. Even in frog 36, the concentration is much too high to be explained as due to contained urine alone.

An objection which can be raised to these experiments is that the urea content of the urine in the kidney may be higher than that of the urine which was collected for analysis just before the organ was removed.

This might be so if the diuresis were rapidly decreasing and the concentration of urea in the urine increasing. To test this point, we determined the course of the diuresis before the experiment by taking three or more periods. When the diuresis is increasing and the concentration of urea decreasing, the same results are obtained. This can best be illustrated by quoting one of our detailed protocols.

Frog 43. Weight 600 grams. April 22, 1924.

Diuresis developed by injection of distilled water into a lymph sac. Anesthetized by injection of 10 cc. of 25 per cent urethane into lymph sac. Ureters cannulated.

1:02–1:07. Urine I, 1.1 cc.
1:07–1:14. Urine II, 1.5 cc.
1:14–1:19. Urine III, 1.05 cc.
1:19–1:26. Urine IV, 1.65 cc.
1:26–1:26:30. Both kidneys removed.
1:27–1:28. Blood drawn from heart.
1:28–1:33. Liver, spleen, stomach and skeletal muscle removed.

Results of the analysis of the blood, kidney and urine IV are given in table 3 under frog 43. The urine specimens gave the following results.

	UREA	AMMONIA (AS UREA)	UREA + AMMONIA
Urine I	17.4	7.8	25.2
Urine II	16.4	7.5	23.9
Urine III	20.2	7.8	28.0
Urine IV	12.7	8.7	21.4

The same method has been used to test whether phenol red is concentrated in the frog's kidney. Diuresis was induced, the dye injected intravenously, and after ten to fifteen minutes, samples of blood, urine and tissues taken for analysis. The results indicate that the kidney contains more phenol red than can be accounted for by the contained urine. Of course, the urine contained in the kidney is less concentrated than the last specimen collected from the ureter. The time at which there is a maximum concentration of the dye in the renal cells will vary with the conditions of the experiment, and hence absolutely consistent results are not to be expected. The dorsal and ventral parts of the kidney were assayed separately, although the relative weights of the two portions varied in different experiments. It seems clear that the dorsal part of the kidney contains more phenol red than the ventral. This difference is discussed later. Frogs 49 and 57 were anesthetized with ether; frog 50 with urethane. All figures represent milligrams per cent (Table 4).

Concentration of urea and phenol red in the cells of the mammalian kidney. Determinations of the urea content of the kidney have been made by Marshall and Davis (16) for the dog, Cushny (20) for the rabbit, and Marshall and Vickers (21) for the dog. These do not support the idea of storage of

urea in the renal cells, as the increased concentrations found can possibly be explained by the relatively concentrated urine unavoidably present in the tubules. Mayrs (5) has recently tested the question in a more direct manner for rabbits by using a method quite similar to the one we have used for the frog. He finds the concentration of urea in the kidney fluid is about the same as that in the plasma. Since the plasma level of urea was very much increased in these experiments by the injection of

TABLE 4

	FROG 49 (APRIL 28)	FROG 50 (APRIL 29)	FROG 57 (MAY 14)
Blood		4.6	1.3
Liver	1.6	1.0	
Heart	0.5	1.9	
Lung	1.0	3.0	
Muscle	Very faint trace	Very faint trace	
Stomach		0.5	
Spleen		Trace	
Kidney, dorsal	25.0	7.3	5.1
Kidney, ventral	11.0	5.4	3.8
Urine	24.0	8.9*	10.0‡

* Urine collected in two-minute periods gave 15.5, 11.4, 8.9 mgm. per cent. Urine in renal tubules is, therefore, of lower concentration than the urine figures indicate. In this experiment dorsal and ventral portions were of equal size, while in others ventral was much larger.

‡ The phenol red was injected into a lymph sac, and the urine collection made about forty minutes later. Urine collected in two nine-minute periods gave 15 and 10 mgm. per cent.

TABLE 5

NUMBER	ANIMAL	MILLIGRAM PER CENT OF UREA IN			
		Blood	Urine	Kidney cortex	Kidney medulla
1	Dog	38	93	42	65
2	Rabbit	39	213	34	85
3	Rabbit	37	83	22	59
4	Rat	115	4790	185	669

urea and sulphate, it is possible that any storage present when the plasma urea is normal might be masked. This objection is considered by Mayrs, but regarded as unlikely. However, in view of our findings with phenol red in the mammal to be considered later and with urea in the frog, it cannot be discarded. We have repeated the experiments on a few animals where the plasma urea was at the normal level, to settle this point, and also to serve as a check on our results with urea in the frog's kidney. Diuresis

has been produced by the injection of 5 per cent sodium sulphate in experiments 1 and 3 and by Ringer's solution in experiment 2. The cortex and medulla were analyzed separately, the intermediate zone being discarded in sampling. From all available evidence on secretion the storage would most probably occur in the cortex, while if no storage occurs the medulla would be expected to contain the greater concentration, as it must contain more urine of higher urea content. The results in table 5 give no evidence of a higher concentration in the cells of the cortex than in the blood, while the medulla content in urea is always lower than the urine and higher than the cortex. In the experiment on the rat, no anesthetic was used, 2 cc. of 5 per cent urea were injected intraperitoneally, the bladder emptied by pressure, and one hour later the animal was killed by decapitation. This experiment will be referred to in another connection. The following protocol gives the details of one of the experiments.

Rabbit anesthetized with urethane. Ureters, external jugular vein and carotid cannulated.

12:02–12:20. Infused 42 cc. of 5 per cent sodium sulphate intravenously.

12:20:30–12:21:30. Urine I from right ureter.

12:21:30–12:22:30. Urine II from right ureter.

12:22:30–12:22:50. Right kidney extirpated.

12:22:30–12:23:00. Blood sample taken.

Urine I contained 83 and urine II 83 mgm. per cent of urea. Samples of cortex and medulla of right kidney analyzed for urea (corrected for ammonia content) with result given in table 5 under experiment 3.

TABLE 6

Phenol red milligrams per cent

BLOOD	URINE	KIDNEY CORTEX	KIDNEY MEDULLA
5.0	14.0	13.5	4.0
2.5	9.0	5.7	1.0

Similar experiments with phenol red in the rabbit indicate that a concentration or storage of this substance occurs in the cortex of the secreting kidney. This was concluded by Marshall and Vickers (2) from their work on the dog from other data, but only demonstrated for the non-secreting kidney. Table 6 gives the results of two experiments.

Relative rate of elimination of urea and phenol red. Ambard (22) in 1910 proposed two laws governing the rate of excretion of urea by the kidney, which were embodied in his now well-known Ambard's constant. That these have not been very satisfactory in predicting the rate of elimination is indicated by the many modifications formulated by subsequent workers. Under certain conditions, however, it has been shown that the rate of

excretion of urea varies directly as the blood urea concentration (16), (23), (28). When a large volume of fluid and a certain amount of urea are taken by mouth, the remarkable constancy of this relation for any one individual has been recently demonstrated by Addis and Drury (24) for blood urea concentrations up to about 100 milligrams per cent. Drury (25) has extended these observations for the rabbit by the injection of urea, and concluded that "the rate of urea excretion continues to increase in direct proportion to increase in blood urea concentration even when the concentration rises to over 700 mg. per 100 cc." This is exactly what

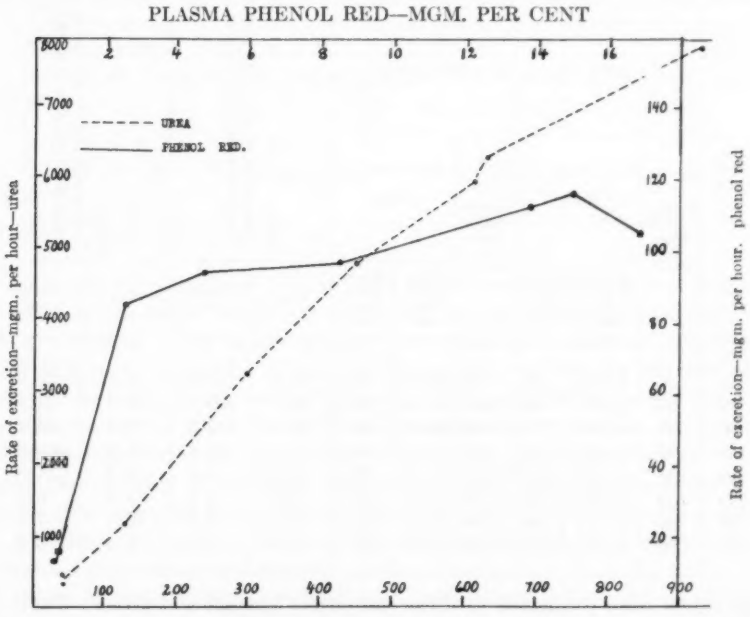

Fig. 1

would be predicted if urea is eliminated entirely by glomerular filtration, provided it is not reabsorbed and the rate of filtration is constant.

Certain scattered observations led us to believe that any direct relationship between the concentration in the blood and the rate of excretion would not be true for phenol red over any extended range of concentration. Of course, on the basis of previous work (2) the excretion rate should depend on the amount stored in the renal cells, which should be proportional to the amount injected or the concentration in the blood. However, it is natural to suppose that at a certain concentration the renal cells would

become saturated with the substance and any further increase in the rate of elimination would be brought about only by the increased amount of the free dye in the glomerular filtrate. The matter was tested as described below.

Diuresis was produced in a dog, and at appropriate intervals injections of a mixture of urea and phenol red were given intravenously. After a suitable time for equilibrium between blood and tissues to be established, samples of blood and urine were collected. Urea and phenolsulphone-

TABLE 7

URINE PER HOUR	UREA			PHENOL RED			RATIO: MILLIGRAM PER CENT IN URINE / MILLIGRAM PER CENT IN PLASMA		RATIO: MILLIGRAM PER HOUR IN URINE / MILLIGRAM PER CENT IN PLASMA	
	Plasma (milligram per cent)	Urine		Plasma (milligram per cent)	Urine					
		Milligram per cent	Milligram per hour		Milligram per cent	Milligram per hour	Urea	Phenol red	Urea	Phenol red
cc.										
20	41	1,775	355	0.64	80.0	16.0	43.1	125.0	8.6	25.0
22	40	2,119	470	0.59	60.9	13.4	53.0	103.1	11.7	22.7
30	127	3,910	1,175	2.54	285.5	85.5	31.0	112.3	9.2	33.7
124	297	2,635	3,285	4.70	75.8	94.0	8.9	16.1	11.0	20.0
238	449	2,018	4,850	8.45	41.1	97.7	4.5	4.9	10.8	11.5
260	612	2,305	6,000	13.80	43.3	112.7	3.8	3.1	9.8	8.2
260	630	2,438	6,340	15.00	44.8	116.7	3.8	3.0	10.1	7.8
242	935	3,254	7,880	16.90	43.2	104.5	3.5	2.5	8.5	6.2

phthalein were estimated in these samples of plasma and urine. Table 7 and figure 1 contain the results. The following protocol gives the details of the experiment.

May 9, 1924. Collie dog, 16.0 kilos. Anesthetized with paraldehyde. Ureters cannulated, leg vein prepared for injection, and carotid artery for bleeding. Urine was collected only from one kidney (left).

10:00–10:10. Injected 100 cc. of 5 per cent sodium chloride.

10:15–10:19. Injected 30 mgm. of phenol red in 50 cc. of 5 per cent sodium chloride.

10:27:00. Sample of blood.

10:27:30–10:30:30. Urine 1.0 cc.

10:31:00. Blood.

10:31:30–10:34:30. Urine 1.1 cc.

10:35–10:36. Injected 90 mgm. of phenol red and 10 grams of urea in 40 cc. of Ringer's solution.

10:43:00. Blood.
10:43:20–10:46:21. Urine 1.5 cc.
10:46:30–10:50:00. Injected 120 mgm. of phenol red and 20 grams of urea in 70 cc. of Ringer's.
10:57:30. Blood.
10:58:00–11:01:00. Urine 6.2 cc.
11:01–11:05. Injected 180 mgm. of phenol red and 20 grams of urea in 80 cc. of Ringer's.
11:12:10. Blood.
11:12:20–11:15:20. Urine 11.9 cc.
11:16–11:23. Injected 240 mgm. of phenol red and 20 grams of urea in 90 cc. of Ringer's.
11:30:00. Blood.
11:30:30–11:33:30. Urine 13.0 cc.
11:34–11:38. Injected 120 mgm. of phenol red and 10 grams of urea in 40 cc. of Ringer's.
11:45:00. Blood.
11:45:30–11:48:30. Urine 13.0 cc.
11:58–12:02. Injected 180 mgm. of phenol red and 36 grams of urea in 120 cc. of Ringer's.
12:09:00. Blood.
12:09:10–12:12:10. Urine 12.1 cc.

The rate of excretion of urea is fairly closely proportional to the plasma concentration, while for phenol red this is true only at relatively low plasma concentrations. The kidney is much more efficient in eliminating phthalein than urea when the plasma levels are low, but exactly the reverse obtains at high plasma concentrations. The gradually increased rate of elimination of the dye at the higher plasma concentrations is of course due to its increased content in the glomerular filtrate. That the introduction into the organism of the large quantities of phthalein has not exerted a toxic effect upon the kidney is probable as its efficiency in eliminating urea is practically the same throughout the experiment. This experiment was repeated on a second animal with similar results.

Since we have shown that both urea and phenol red are stored in the renal cells of the frog, the curves constructed for these bodies might both be expected to resemble that of the latter for the dog. About six experiments of this nature have been carried out on frogs. Several difficulties arise, however, in the interpretation of these curves for the frog. In the mammal, it is fairly well established that the elimination of phthalein is independent of the urine volume (26), (27) and that this is true of urea when the urine volume exceeds a certain minimum (12), (28). Data on these points are lacking for the frog, but from our own experiments we believe that the rate of elimination of both urea and phthalein are markedly influenced by the rate of eliminaton of water. Therefore, only periods where the urine volume is about the same should be compared, or allowance made for changes in water excretion Again, the amount of blood

withdrawn from the frog may quite conceivably produce an anemia sufficient to affect the kidney. The frog's blood volume has been estimated at about 3.5 to 6.2 per cent of the body weight (29) and the total amount of blood taken in our experiments has varied from 1.0 to 1.5 per cent of the body weight (16 to 44 per cent of the estimated blood volume). Experiments have been extended over two or three days with the withdrawal of only one or two small samples of blood each day. No essential difference in the results was noticed from those of experiments where four samples were taken in the course of a single day. To obtain sufficient urine for analyses, the periods of collection must be made much longer in the frog than in the dog, which means that the blood and urine samples may not give such exact comparative points. Another difficulty, which was only realized after several experiments had been performed, is the fact that large doses of phenolsulphonephthalein are toxic for frogs, producing delayed convulsions (30) similar to those of acid fuchsin (31). The experiment on frog 60 was carried out using urea alone for this reason. This objection, however, only applies to the final periods of the experiments. Owing to these difficulties and in view of the fact that more than four points have never been obtained on any one frog, we give the results only in the form of tables. These results seem to resemble both for urea and phthalein in the frog those obtained for the latter only in the dog.

All experiments of this type on the frog were carried out without anesthesia. Injections of urea and phenol red were made into a lymph sac. A period was not started until at least 30 minutes after injection. Urine was taken for a period of five to thirty minutes, blood being taken at the end of the period. Whole blood was used for both urea and phthalein estimations. The urea figures for plasma are about the same as for whole blood. Phenol red, however, is present to the greater extent, if not entirely, in plasma. Therefore, the figures for plasma would be considerably higher than those given in the tables, and the ratios smaller. On the other hand, a single experiment indicated that phenol red is only about half as concentrated in the ultrafiltrate (2) as in plasma (where the concentration was 2 mgm. per cent). If the concentration of the ultrafiltrate of plasma is used for comparison the ratios given would be nearly correct. The following three tables (8, 9 and 10) are summaries of representative experiments.

If the data in these tables for the frog are compared with similar data given in table 7 for the dog, certain definite differences are to be observed. The concentration ratios for urea and phenol red are about the same for the frog; but for the dog, phenol red is concentrated much more than urea when the plasma concentrations are low, but exactly the opposite is true when the plasma concentrations are high. The concentration ratios for urea vary inversely as the volume of urine and never fall below 3.5 for the dog, while in the frog the ratios tend to approach unity as the

TABLE 8

Frog 54

URINE PER HOUR	UREA			PHENOL RED			RATIO: MILLIGRAM PER CENT IN URINE / MILLIGRAM PER CENT IN BLOOD		RATIO: MILLIGRAM PER HOUR IN URINE / MILLIGRAM PER CENT IN BLOOD	
	Blood (milligram per cent)	Urine		Blood (milligram per cent)	Urine					
		Milligram per cent	Milligram per hour		Milligram per cent	Milligram per hour	Urea	Phenol red	Urea	Phenol red
cc.										
8.2	6.3	25	2.0	0.75	2.73	0.22	4.0	3.6	0.32	0.29
21.8	22	35	7.6	2.40	5.00	1.09	1.6	2.1	0.35	0.45
33.0	108	125	41.2	4.03	5.87	1.98	1.2	1.5	0.38	0.49
20.0	227	267	53.4	6.45	8.40	1.68	1.2	1.3	0.23	0.26

TABLE 9

Frog 56

URINE PER HOUR	UREA			PHENOL RED			RATIO: MILLIGRAM PER CENT IN URINE / MILLIGRAM PER CENT IN BLOOD		RATIO: MILLIGRAM PER HOUR IN URINE / MILLIGRAM PER CENT IN BLOOD	
	Blood (milligram per cent)	Urine		Blood (milligram per cent)	Urine					
		Milligram per cent	Milligram per hour		Milligram per cent	Milligram per hour	Urea	Phenol red	Urea	Phenol red
cc.										
6.1	33	79	5	1.45	4.3	0.26	2.4	2.9	0.15	0.18
21.5	65	75	16	2.40	8.4	1.80	1.1	3.5	0.25	0.75
19.0	259	281	53	5.10	9.6	1.83	1.1	1.9	0.20	0.36
9.6	496	482	46	7.40	10.0	0.96	1.0	1.3	0.093	0.13

concentration of urea in the plasma rises. Again, in the mammal, the ratio $\frac{\text{mgm. per hour in urine}}{\text{mgm. per cent in plasma}}$ for phenol red shows first a slight increase and then a steady fall as the plasma concentration rises, while the same ratios for urea are nearly constant over all ranges of plasma concentrations. In the frog, the above ratios for both urea and phenol red are similar to those of the latter for the dog. The increase in the ratio of rate of excretion to plasma concentration observed for phenol red in the dog and both substances in the frog would appear to indicate that a slight rise of

TABLE 10

Frog 60

URINE PER HOUR	UREA			RATIO: MILLIGRAM PER CENT IN URINE / MILLIGRAM PER CENT IN BLOOD	RATIO: MILLIGRAM PER HOUR IN URINE / MILLIGRAM PER CENT IN BLOOD
	Blood (milligram per cent)	Urine			
		Milligram per cent	Milligram per hour		
cc.					
9.2	3.6	24	2.2	6.6	0.612
9.6	3.3	34	3.2	10.3	0.970
12.0	117	173	20.8	1.48	0.186
16.8	364	420	70.7	1.15	0.194
15.0	838	1004	151.0	1.20	0.180

plasma concentration acts as a stimulus to the secreting cells. More data and observations are needed on this point.

Certain observations, concerning which we do not desire to report at present, suggest that the secretion of urea by the frog varies markedly in amount under different conditions. De Haan and Bakker (32) report that certain dyes are much more quickly eliminated in the summer than in the winter frog. Van der Heyde states that pigment is much more abundant in the urine of frogs kept at a high temperature than in those at a low one (33), (34). Possibly the urinary pigment is secreted from the store of yellow pigment seen in the dorsal cells.

Discussion. The facts which have been reported concerning the excretion of urea and phenolsulphonephthalein would appear to indicate that both of these substances are secreted by the tubules of the frog's kidney, but that only phenol red is secreted by the mammalian kidney, urea being eliminated entirely by filtration. The evidence in support of this may be briefly summarized as follows.

1. The determination of the concentration ratios for various substances in the frog indicates that this ratio for urea is much greater than those for other bodies. Phenol red resembles urea in this respect. In the mammal, on the other hand, urea is not concentrated more than many other substances, while the concentration ratio for phenol red is greater than that of urea. These statements in regard to concentration ratios are only true under certain conditions. In the frog, when the plasma content of urea is greatly increased by injection, and when large amounts of phenol red are given neither is concentrated to any extent by the kidney and hence the concentration ratios do not probably surpass those of other substances. In the mammal, when the relative concentration ratios for urea and phenol red are compared at different plasma levels, it is found that with relatively low concentrations the latter has a much greater ratio, while at high concentrations, the ratio for urea may be the greater. This indicates that the determination of concentration ratios should be made for various concentrations of the substances in the plasma to be interpreted properly.

Similar discrepancies in concentration ratios of various substances under different conditions are noticed in the literature already mentioned. Mayrs (14) finds sulphate to be concentrated about 1.5 to 2.7 times more than urea, but in a later communication (5) on the excretion of urea and sulphate calculations from his data show both to be concentrated to about the same extent. The conditions of the experiments were somewhat different. Underhill (8) finds in two experiments phosphate to be concentrated to the same extent as urea, but in two others the concentration is about twice as great. The differences which White (7) has found in dogs depending on whether or not the plasma level is raised have already been discussed.

2. Both urea and phenol red occur in a much greater concentration in the cells of the renal tubules of the frog than in the blood and other tissues (with the possible exception of the liver for urea). This is quite similar to the concentration of phenol red in the convoluted tubules of the mammalian kidney which is believed to be a stage in the secretion of the dye. When the cortex of the mammalian kidney is examined for urea no storage in the convoluted tubules can be demonstrated, the concentration being about the same as plasma and much lower than that of the medulla or urine. The amount of urea in the medulla is what one might expect if urea occurs in the cells in concentration equal to the plasma and a certain amount of urine is contained in the tubules. When the content of phenol red in cortex and medulla is compared under the same conditions, the cortex is found to contain more than the medulla and much more than can be accounted for by the urine contained in the tubules.

The dorsal part of the frog's kidney contains more urea and phenol red than an equal weight of the ventral portion. The structure of this kidney as determined by Nussbaum (35) and Huber (36) indicates that the glomeruli and distal or second convoluted tubules are confined mainly to the ventral portion and the proximal or first (containing the cells with brush border) to the dorsal part.[4] This suggests that it is mainly if not entirely the cells of the proximal or first convolution which secrete these substances

3. When the efficiency of the mammalian kidney for eliminating urea is determined over a wide range of plasma concentrations (the urinary volume being fairly large), it is found to be nearly constant. With phenol red, on the other hand, the efficiency is at first greater than with urea but steadily decreases as the plasma concentration of this substance is increased. This is interpreted as being due to the secreting cells becoming saturated with the dye.[5] In the case of the frog's kidney, urea and phenol red are both less efficiently eliminated at high plasma concentrations than at low ones. Filtration alone, of course, is less efficient than filtration and secretion combined.

Our data do not exclude the possibility that urea is secreted by the mammalian kidney without being concentrated in the cells. The process would then be entirely different from that of the secretion of urea or phenol red in the frog or phenol red in the mammal. The fact that concentration or storage of a substance occurs in the renal cells as a preliminary to secretion would seem to bring secretion by the kidney more into line with what is known about secretion in the case of other glands. Thus, in the case of the submaxillary gland the mucin of the saliva is secreted from a store which is present in the cells. Although we believe the possibility of the secretion of urea by the mammal without concentration in the cells is a rather unlikely hypothesis, it cannot be entirely eliminated at present. It is rendered quite unlikely, however, by the following considerations.

1. The fact that bicarbonate is concentrated about as well as urea when the concentration of the latter is normal, but that of the former is raised in the blood indicates that if urea is secreted, bicarbonate is also. That a similar argument can be drawn for White's (7) results on urea and phos-

[4] More accurate information is needed concerning the structure of the frog's kidney. From examination of serial sections it would appear that about two-thirds or more of this organ consists of proximal convoluted tubules. The dorsal portion contains mainly these tubules, while the ventral part contains nearly as many proximal as distal tubules. The differences in the content of urea and phenol red in the dorsal and ventral parts of the kidney would be much more exaggerated if a clean cut separation of proximal and distal tubules could be made.

[5] Ultrafiltration of plasma containing the highest concentration of phenol red encountered in these experiments showed that the concentration in the filtrate was 50 per cent as compared with about 35 per cent of that from the plasma of the lower concentrations.

phate has already been mentioned. This necessitates the idea of both reabsorption and secretion of the same substance, and means that practically all substances are secreted as well as filtered.

2. The almost exact proportionality of the rate of urea excretion to the concentration in the plasma under certain conditions is what might be expected on the hypothesis of filtration alone. It would seem less likely on the basis of filtration and secretion. However, since in considering the secretion of urea without storage in the renal cells we are dealing with an entirely unknown possibility nothing can be predicted about it with certainty.

3. No satisfactory data which would prove the secretion of urea by the mammalian kidney have been obtained. Numerous workers have, however, obtained results which they believe established the secretion of urea by the mammal. An examination of the available evidence for the secretion of urea by the mammalian kidney shows it to be quite inconclusive. In our last paper on the effect of temporary anemia on the kidney, we believed that the most satisfactory interpretation of our results was by assuming the secretion of urea, but frankly admitted the possibility of explaining the results on filtration and reabsorption alone (4). In view of the further data presented here and the great dissimilarity in the elimination of urea and phenol red little weight can be attached to our previous interpretation. The experiments of White (7) and Underhill (8) on concentration ratios already discussed offer no direct proof of secretion. The latter interprets his results on urea, phosphate and creatinine as being incompatible with Cushny's "filtration reabsorption" hypothesis in its present form, and concludes that one or more of these substances must be actively secreted. White (7) has utilized another method to obtain evidence of the secretion of urea, sugar, phosphate and sulphate by the tubular cells. It depends on the assumptions that if the rate of urine flow from the ureter is increased in one period compared to another of any given experiment, the rate of glomerular filtration is increased or at least not reduced in the period of greater urine flow; and, that when the rate of urine flow is greater in one period than in another the reabsorption of any body must be less in the case of the period of lesser urine flow. We do not believe that either of these assumptions is necessarily true; and as no evidence for their validity has been presented, arguments based upon them do not prove the secretion of urea (or the other substances considered). At different times attempts have been made to determine the secretion of urea by seeking for a localized concentration of this substance in the renal cells by micro-chemical methods. Leschke (37) claims to have demonstrated localized concentration of urea (as well as uric acid, chloride and phosphate) in the epithelium of the convoluted tubules. Oliver (38) has confirmed his observation for urea, but admits that the reaction used is

not specific for the purpose, and Walter (39) is still more doubtful of its value. Policard (40) using the more specific xanthydrol reagent of Fosse (41) could not find any precipitate of urea in any tubule cells; but Chevallier and Chabanier (42), with the same method, detected urea in the cells of the convoluted tubules, all blood vessels of the kidney, and the lumina of the ducts of Bellini. This method has been subsequently employed by Oliver (43), Stubel (44), Piras (45), Walter (39) and Hollman (46). Their findings while differing from one another in some minor details seem to confirm Chevalier and Chabanier in localizing the crystals of dixanthyl urea in the epithelium of the convoluted tubules, the lumina of all tubules including the glomerular capsule, the tissue spaces between the tubules, and the lumen of the blood vessels. Using the liver as a control, Oliver and Piras find the precipitate only in the blood vessels and not in the hepatic cells, while Walter states that it is to be seen in both. All of these observers conclude that their data prove the secretion of urea by the cells of the convoluted tubules, while Walter adds that his results disprove the filtration theory of the glomerulus as this structure must also actively secrete urea. It seems to us obvious, however, that the conditions under which the precipitation with xanthydrol occurs must be more carefully studied before any conclusions can be drawn from this type of experiment. Oliver assumes that there is a threshold in cellular protoplasm below which no precipitate is formed, but that such a threshold (or at least as high a one) does not exist for blood or partially elaborated urine. The best results were obtained when the urea concentration in the urine was increased by protein feeding or injection of urea. Possible explanations of the results without assuming a localized concentration of urea in the cells of the convoluted tubules would be the existence of substances inhibiting the precipitation in the renal cells other than those of the convoluted tubules and the cells of the liver, or that the urea has diffused from its more concentrated solution in the lumen into the cells post mortem. It is difficult, however, on the latter assumption to see why the cells of the medullary tubules should not also show the presence of urea. The experiments of Mayrs give no support of the idea of a concentration of urea in any cells of the renal tubules, and the data given in table 5 show that no accumulation of urea can be detected by chemical analysis of the cortex (convoluted tubules). We have repeated one of Oliver's experiments determining the urea content of the cortex and medulla chemically instead of relying on histochemical reactions. This experiment is the one given for the rat in table 5. As already pointed out, it shows clearly that urea is not stored or concentrated in the cortex and hence is not in the convoluted tubules.

The experiments recently published by Bieter and Hirschfelder (9) on the elimination of phenol red by the frog led them to the conclusion

that there is no storage or secretion of this substance by the tubule cells. This is directly opposed to the evidence of Marshall and Vickers (2) of the secretion of this dye by the mammalian kidney and also to the conclusions concerning the excretion of this substance in both the frog and mammal given in this paper. The apparent discrepancy is, however, easily explained by the fact that concentration and secretion of phenol red takes place only in the proximal convoluted tubules of the frog, and that Bieter and Hirschfelder apparently observed only the ventral surface where no proximal convolutions can be seen. This matter is discussed in the following paper by Edwards and Marshall (47).

In this paper, the question of secretion of only urea and phenol red by the amphibian and mammalian kidneys has been considered. It is natural to enquire how far the conclusions reached in regard to these substances will apply to other constituents of the urine. From the results obtained, we feel that each substance must be considered separately in regard to its secretion, and data obtained on one class of animals cannot be necessarily transferred to another. It will be of great interest to apply to different urinary constituents in various classes of vertebrates what we may call two criteria for secretion; namely, the concentration of a substance in the renal cells, and the behavior of the ratios of rate of excretion to plasma concentration at different plasma levels. The data, for instance, presented by Mayrs (6) in favor of the secretion of uric acid by the kidney of the fowl would be greatly strengthened by the finding of a concentration of this body in the bird's kidney and by a study of its excretion curve compared to that of phenol red and urea. The question of the elimination of urinary constituents other than urea by the frog's kidney will be discussed in a later communication. It might be mentioned that the inability of the kidney of this animal to concentrate chlorides or bicarbonate is probably related to the absence of the loop of Henle, although further work on different classes of vertebrates will be necessary in order to reach a final conclusion.

Phenol red is the only substance for which we believe definite proof of secretion by the tubules of the mammalian kidney has been obtained. As stated in a previous communication, it is extremely likely that some of the normal urinary constituents are secreted by a similar process. Urea is not secreted, or at least not by the same mechanism as phenol red. The fact that ammonia is formed in the kidney (48) suggests that it is secreted, and the same may be true for creatinine. It is interesting to recall that these three substances, phenol red, ammonia, and creatinine, differ from other substances in that their rate of elimination is not affected by increase of blood flow or moderate decrease of the same through the kidney (27), (49). It is also possible that many other substances occurring in the blood in small amounts and relatively toxic for the organism are secreted,

as this would give a more rapid elimination than filtration alone. However, much more data will have to be obtained before decision on these questions can be made.

The question of the secretion of water by the tubules naturally arises for consideration. Are the substances which are eliminated by this route added to the fluid in the lumen by some process not involving the transfer of fluid, or is the secretion similar to that of the true secretory glands in being a hypotonic solution of blood constituents? The experiment of Nussbaum showing that when the renal arteries of the frog are ligated no urine is spontaneously eliminated although the tubules are still supplied with blood by the renal portal vein, is generally given as evidence that the glomeruli are the chief site of fluid elimination. Since this is supported by much less direct evidence, the question has generally been regarded as settled. The idea of some fluid being eliminated by the tubules cannot be, however, disproven at present. In fact, the elimination of fluid by frogs, with ligated renal artery, after the injection of urea is held by many as evidence that the tubules secrete urea in solution when stimulated by an excess of this substance in the blood. The difficulties attending an acceptance of this evidence have been discussed by Cushny (50). A repetition of the "Nussbaum experiment" with careful analyses, by modern micromethods, of the blood and bladder fluid obtained would seem desirable.

SUMMARY AND CONCLUSIONS

Experiments have been undertaken on the elimination of phenol red and urea by the amphibian and mammalian kidney. The observations of Marshall and Vickers for the secretion of phenol red by the mammal have been extended and found to hold for the frog's kidney. Phenol red is concentrated or stored in the cells of both the amphibian and mammalian kidneys. The efficiency of the kidneys of both classes of animals is much lower for this body when the plasma concentrations are high than at lower ones. This is taken to mean a saturation of the secreting cells. Urea is concentrated in the frog's kidney in a similar way to phenol red, but no evidence of such concentration in the cells can be obtained for the mammalian kidney. Also, the efficiency of elimination of urea is practically the same at all plasma levels in the mammal, while in the frog its behavior is different and similar to that of phenol red.

It is concluded from these observations that phenol red is secreted by both the amphibian and mammalian kidney, while urea is secreted by the former. If it is secreted by the latter the process of secretion does not involve a preliminary concentration in the renal cells, and differs from that of the secretion of urea in the frog and of dyes in both classes. Since no satisfactory evidence from the literature has been found for the secretion

of urea in the mammal but rather the reverse, it is probably eliminated only by filtration. This, at least, is the best working hypothesis at present.

The fact that both urea and phenol red are more concentrated in the dorsal part of the frog's kidney than in the ventral, taken in connection with what is known of the structure of this kidney leads to the conclusion that these substances are secreted mainly, if not entirely, by the proximal convoluted tubules.

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MICROSCOPIC OBSERVATIONS OF THE LIVING KIDNEY AFTER THE INJECTION OF PHENOLSULPHONEPHTHALEIN

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As the result of experiments (1) on the secretion of phenolsulphonephthalein (phenol red) by the dog's kidney, the possibility was suggested of obtaining additional evidence of the secretion of dyes by microscopic observation of the kidney in the living animal. The recent publication of Bieter and Hirschfelder (2), using a similar method, on the elimination of phenol red and indigo carmine has led us to publish certain of our observations on the former substance. The conclusion of these investigators is not in accord with those obtained by using other methods (1), (17). They also differ from the conclusion to which we have been led by experiments the results of which are briefly presented in this paper. More detailed observations which have been obtained with a series of dyes are reserved for a future communication.

But few observations are recorded in the literature of attempts to study microscopically the kidney of living animals. Nussbaum (3) observed the kidney of living salamanders (*Triton cristatus*) to determine whether a back flow through the glomeruli takes place when the renal arteries are ligated. He states that he attempted to study the elimination of indigo carmine under these conditions, but without success. The observation that the lumina of the tubules become widened with increased urine flow is, however, recorded. Cohnheim (4), who observed the kidneys of two heteropods after the injection of dyestuffs into the body cavity, concluded that the dyes are stored in this organ and bound by the protoplasm as salts.

In 1912, Ghiron published a method for the microscopic observation of the kidney in the living mouse (5), and subsequently reported the results of his observations on the elimination of certain dyes (6). He claims to have seen the dye appear first in the lumen, then pass from lumen to cell, and finally accumulate in the outer border. A discussion of his results is reserved for another publication since the dyes with which he worked differ from phenol red and are excreted to only a small extent by the kidney. Khanolkar (7) is the only investigator, as far as we know, who has attempted to repeat Ghiron's experiments. He states "owing to the thickness of the capsule, and the presence of pigment in the mammalian kidneys, it was found impossible to repeat these observations on mice, and I was unable to confirm Ghiron's statements."

Recently Richards and his co-workers (8) (9) (10) have carried out an intensive study of the frog's kidney by direct observation, but it has been thus far confined to

glomerular function.[1] Hill (11) has studied the glomerular circulation in the frog's kidney in an effort to measure the capillary glomerular pressure.

The methods which we have used were as follows. Frogs (*Rana pipiens*) weighing from 15 to 40 grams and white rats weighing about 100 to 150 grams were used. Both were anesthetized by the injection of 20 per cent urethane (0.5 to 1.0 cc. for the frog, and 0.6 to 1.5 cc. for the rat). All operative procedures were done with a thermocautery in order to minimize hemorrhage. In exposing the kidney of the frog, an incision was made to one side of the mid ventral line, the abdominal vein was doubly ligated and cut, and a V-shaped flap made from the fore to hind limbs. Parts of the viscera were either deflected or removed if necessary. The peritoneum along the outer margin of the right kidney was then cut and a ligature attached to this cut surface. By means of this ligature, gentle traction could be exerted on the kidney when the ventral surface was to be observed, or the organ could be turned over and kept in position to observe the dorsal surface. The frog was immersed in Ringer's solution in a paraffined tray, which could be placed on the stage of the microscope. The lateral margins of the middle portion of the ventral and the mid-dorsal surface were used for all observations. An incision in the linea alba was made in the rat, the wound enlarged by a cross incision through the abdominal muscles, and the intestines packed off with cotton.[2] The kidney was now partially decapsulated and made sufficiently immobile for accurate observations by sticking pins through the torn edges of the capsule, and elsewhere in the body wall around its outer surface. The cotton packs covering the deflected viscera were held in place by special pins. The rat's kidney was always somewhat in motion due to respiration or arterial pulsation. The peritoneal cavity was next filled with warm Ringer's solution. Cannulae were inserted into the jugular vein for injections, and into the bladder in order to observe urine flow and collect specimens. The animal was immediately placed in a box heated to 37°C. when the operative procedures were finished. All observations on the mammal were made with the animal and microscope in this box. Illumination was provided for by means of reflected light from an arc lamp. The light was passed through

[1] In 1921, microscopic observation of the kidney of the living frog was begun by one of us. It was not known at that time that Richards had been engaged in similar studies. The interest in these earlier observations was mainly concerning the glomeruli, but when at New Haven in 1922, it became known that Richards and his coworkers had advanced much farther along this line the work was discontinued. A study on the secretion of phenol red by the dog's kidney led to the use of the microscope for certain observations of the tubule.

[2] In our early experiments all of the abdominal viscera with the exception of the kidneys and liver were removed.

either a saturated solution of alum or a dilute solution of methylene blue, and then condensed on the kidney by means of a lens. The objectives used were a 16 mm. Spencer achromatic and a Zeiss D star water immersion. These with number 10 eye pieces on a mon-objective binocular microscope gave magnifications of 185 and 740 diameters respectively.

A number of experiments have been carried out on the frog. In some only the ventral surface was observed, in others only the dorsal side, while in the majority both sides were utilized by turning the kidney over at intervals by means of the ligature mentioned above. Although the individual experiments have differed from one another in certain details, the points which have been selected for description here have been very consistent in all the observations.

One-tenth to four-tenths cubic centimeter (1 cc. = 6 mgm.) of phenol red is injected intraventricularly into a frog while the kidney is being observed. A distinct reddish blush is seen on the ventral surface. This disappears immediately after the injection. Within a few minutes the visible glomerular capsules are seen to contain the dye, but the tubules appear unchanged in color from the normal. No indication of dye can be observed in either the cells or lumina. In a variable time, depending on the rate of urine elimination, the lumina of the tubules contain the dye. These frequently stand out as well defined streaks. The color may be either pink or yellow or of any intermediate shade. The experiment was always discontinued before the excretion was complete. Throughout all the experiments the cells of the ventral tubules have appeared to contain little if any of the phenol red.

Observation of the dorsal surface, however, shows an entirely different picture. Immediately after injection, there is the pinkish blush, and within a few minutes the cells of the tubules definitely contain the dye. These cells normally contain a yellow pigment, but the change in color is quite easily noted by continuous observation before, during, and after the injection. As far as we have observed the appearance of the dye in the cells is homogeneous No particular granules or discrete particles are stained by it. The lumen becomes colored (pink, yellow or intermediate) at about the same time as the cells, although in one or two experiments the coloring of the cells seemed to precede any color in the lumen by a very short interval. Very little stress is laid at present on this difference in color owing to the possibility of confusing the color that may be in the lumen with that transmitted by looking through the renal cells. The intensity of color in the cells appears to increase up to 15 minutes after injection. The color in the lumen increases in intensity, and is usually of a slightly or markedly different tint from that of the cells.

In unanesthetized frogs the injection of 0.6 mgm. of phenol red into a lymph sac is followed by the elimination of 17 to 50 per cent of the amount injected in 2 to 4

hours, and 50 to 90 per cent in 24 hours. According to de Haan and Bakker (15), there is a seasonal variation in the ability of the frog's kidney to eliminate dyes. Our observations were made in December and January, the frogs being kept at 13 to 18°C. before, and at room temperature (about 18 to 22°C.) during the observations.

The structure and relation of the tubules of the frog's kidney have been described as follows (12), (13), (14). The first segment is the ciliated neck joining the glomerular capsule to the second segment or proximal convolution. This convolution is larger than the remainder of the tubule, and has cells containing many granules and vacuoles. These are lined with a brush border. The third segment is short and narrow and possesses ciliated epithelium similar to that of the first. The cells are low and contain no granules, vacuoles or mitochondria. The cells of the fourth segment or distal convolution are the so-called rodded epithelium, but possess no brush border. The fifth segment connects the last to the collecting duct, and the cells are cylindrical or cubical. The glomeruli lie in *Rana fusca* equally through the whole organ in three or four irregular rows. In *Rana esculenta*, however, they lie very near the ventral surface of the kidney, in a bow-shaped plane with concave side towards the ventral surface, and are themselves overlaid toward the ventral surface by the fourth segment of the tubules. On the lateral edge of the kidney isolated glomeruli come close under the peritoneum. In *Rana pipiens* and *catesbiana* the arrangement appears to resemble that in *esculenta*. From studies made thus far in these two species, it appears that only proximal convolutions can be seen on the dorsal surface, and distal convolutions on the ventral surface. Hence, our data obtained from the latter side apply to the fourth segment or distal convolution, while those obtained from a dorsal view relate only to the second segment or proximal convolution.

The surface of the decapsulated kidney of the rat was observed before, during, and after the injection of phenol red into the jugular vein. According to reconstructions of the mammalian kidney especially that of Traut (14) it seems evident that only proximal convoluted tubules (first convolutions) and a few junctional tubules are to be seen on the surface. The distal convolutions and glomeruli are too deep to be visible. The accompanying drawing (fig. 1) gives a typical view of what may be seen under these conditions. The amount of phenol red injected varied from 0.4 to 1.0 cc. (1. cc. = 6 mgm.). Frequently 1 cc. of 6 per cent sodium sulphate was given to produce a good flow of urine. About 40 to 60 per cent of the phenol red injected was excreted by both kidneys in 2 hours under the conditions of the experiments.

Immediately after the injection, the whole kidney becomes reddish. The lumen appears distinctly pink during injection and the color fades

immediately after the injection is completed. As soon as this initial reddish blush has disappeared the cells are yellow. This yellow tint increases in intensity for a period of about ten minutes. The color in the lumen is usually of a different tint from that of the cells, although the fact that observations must be made through a layer of cells somewhat obviates any very definite conclusion. The color in the cells is maintained

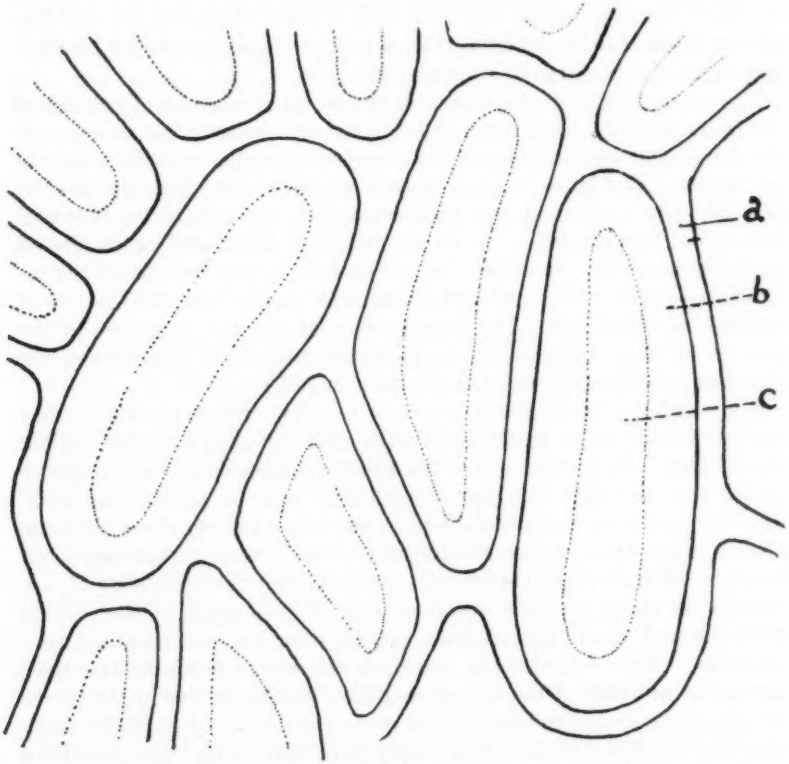

Fig. 1. A combination camera lucida and free-hand outline drawing of a part of a microscopic field of the kidney of a living rat. *a*, capillary; *b*, area occupied by the cells of a convoluted tubule; *c*, the lumen of a convoluted tubule. × 740.

in intensity or at least appears to be but little altered for a period of 1 or 2 hours. Then, there seems to be less dye present in the cells. In from 2 to 4 hours the cells appear to be entirely or almost free of phenol red. At this time only a trace can be detected in the urine. The color in the lumen may be pink, yellow or any intermediate color. The intensity of color or even quality may not be the same in all the lumina observed. Certain tubules seem to have the dye in a much more con-

centrated form than others. These are usually few in number and may be junctional tubules. The cells of these tubules appear smaller, and seem to contain little if any dye. The widening of the lumina of the tubules after the injection of a diuretic has been frequently observed.

The cells in other tissues (liver, spleen, pancreas, muscle, intestine) which can be observed microscopically in the living animal never show in either the frog or the rat more than a trace of phenol red and the picture is quite different from that of the proximal convoluted tubules, where the dye is certainly concentrated.

The accumulation of phenol red in the proximal convoluted tubules of the frog's and rat's kidney can be explained as a stage in the secretion of the dye by this segment, or as a reabsorption of the dye from the glomerular filtrate. The latter is unlikely as it would require that the dye be first filtered, reabsorbed and concentrated in the cells of the proximal convoluted tubules, taken up by the lymph or blood and again filtered by the glomerulus. It is also hard to explain on this hypothesis the relationship between the intensity of the color in the cells and the rate of elimination of the dye. The hypothesis that the dye is present in the cells on its way to secretion is in agreement with other independent observations obtained by different methods (1), (17).

The conclusion given above, it is to be noted, is directly opposed to that recently arrived at by Bieter and Hirschfelder (2) using a similar method for the frog. They believe that the tubules neither secrete nor reabsorb phenol red, but that this dye is eliminated entirely by the glomeruli. The description of the picture seen in the frog's kidney given by these observers has been frequently observed on the *ventral surface alone*, but is not at all true when the *dorsal surface* is also investigated. It appears that these authors observed only the ventral surface, where glomeruli and distal convolutions can be seen, but not proximal convolutions. Their experiments involving the cauterization of the renal arteries at one pole of the kidney and then finding no dye in the lumen or cells of the *ventral* tubules, do not prove the dye is not normally eliminated by the *dorsal* tubules, but simply show that under these conditions no fluid is eliminated.

We realize fully the great difficulties of observation and risks of subjective impression in this type of experiment, but feel that the limited data from which conclusions have been drawn can be easily verified. There are numerous other points which we do not desire to stress at present. The distinct yellow color of the phenol red in the cells would seem to suggest that their protoplasm is of a much greater H-ion concentration than the blood, and hence that acid is possibly secreted by them. However, the fact that the neutrality point may be different in protoplasm than in water and that the salt and colloid effects on the color of

the indicator must be considered, prevents any definite conclusion being drawn. Stieglitz's (18) idea that the reaction of the tubule cells is always opposite to that of the urine can easily be tested on the kidney of the living animal by this method. We prefer, however, to accumulate more data and use other indicators before discussing it.

SUMMARY AND CONCLUSIONS

Direct microscopic observations of the kidneys of living frogs and rats after the intravascular injection of phenolsulphonephthalein show that this substance is present in high concentration in the cells of the proximal convoluted tubules. In the rat, only proximal convolutions can be observed; while in the frog, the dye is to be seen in lumina but not in the cells of the distal convolutions. These results are in accord with the idea that phenolsulphonephthalein is actively secreted by the proximal convoluted tubules of the kidneys.

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PHYSIOLOGICAL REACTIONS AND STRUCTURE OF THE VOCAL APPARATUS OF THE CALIFORNIA SINGING FISH, PORICHTHYS NOTATUS

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The California "singing fish," Porichthys notatus, spawns at tide water off the rocky shores of Monterey Bay. The spawning season is June and July. At this time nests are made in cavities under rather large rocks in the tide pools where fish are often found guarding the eggs which have been deposited on the under surface of the rock which forms the roof of the nest. These specimens are always males, not females. One method of locating them is to walk over the exposed rocks at low tide, depending on the fact that when one steps on the nest rock the disturbance calls forth a protesting sound from the guard beneath. The sound of the paternal fish is produced by a mechanism within the air bladder. This vocalizing power has been known to scientists since the discovery of the genus. It is responsible for many of the local fisherman names that have been applied to the fish, including the one used above.

The air bladder of Porichthys notatus is developed by an evagination from the pharynx comparatively early in the embryological history. Long before the young fish have absorbed the yolk and are set free from their parental spawning rock, the sac is completely closed off from the esophagus and the air-bladder is entirely free in the body cavity. In the adult it is U-shaped. The arms of the U are arranged horizontally with reference to the position of the fish, the limbs extending anteriorly. The wall of the cavity is a thick connective tissue sheath apparently without smooth muscle in its structure. The air-bladder itself has two well-developed muscles of the striated tissue variety. These are located on the outer side of each of the two limbs. The muscles are rather heavy masses, the fibers extending around but somewhat obliquely in the dorso-ventral direction (see fig. 1). The muscles are innervated by short and heavy branches of the vagus nerves. The nerve on each side enters the corresponding muscle at its anterior point and is distributed down through the entire muscle. The lining of the air-bladder is of epithelial tissue. Near the posterior end, that is near the base of the U, figure 2, a transverse membranous diaphragm separates the cavity into two divisions. The greater volume of space

is in the anterior division and it is on the outer walls of the two communicating limbs of the anterior cavity that the muscles are placed, figures 2 and 3. In the center of the diaphragm is a tiny opening into the posterior chamber, not figured. It is not determined certainly whether this diaphragm has any smooth muscle, though some of our gross histological

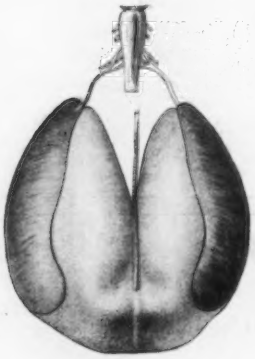

Fig. 1

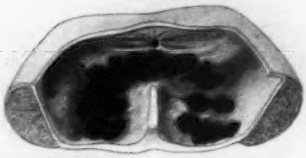

Fig. 2

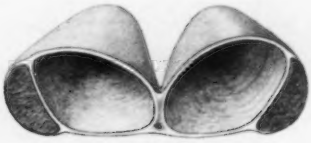

Fig. 3

Fig. 1. Ventral view of the air bladder of Porichthys notatus. The ventral air bladder artery is shown entering the wall at about its median surface. It supplies the gas glands. The two large lateral air bladder muscles are clearly outlined. The branch of the vagus innervating each enters at the most anterior end of the muscle. The ventral surface of the medulla is outlined to orient the vagal relations. Reproduced natural size.[1]

Fig. 2. A cross section of the air bladder to show the diaphragm with its central opening, and the numerous places of the gas glands of the ventral floor of the anterior chamber. Viewed from the antero-dorsal aspect. A part of the dorsal wall and of the median septum is removed. Natural size.

Fig. 3. Cross section of the anterior chamber to show the cavities of the anterior horns and the relative size of the air bladder muscles. Postero-dorsal view, natural size.

preparations indicate that it has. At any rate, the size of the opening varies in different prepared examples in such a way as to suggest variation at different times in the same fish.

The noise or vocalization is produced by sudden unequal pressure on the gases of the two chambers. This sets the diaphragm into vibration. The change in pressure is accomplished by the contractions of the air-bladder

[1] We are indebted to Mr. Geo. T. Kline, Biological Artist of the University of Missouri, for drawings 1 and 3, and to Prof. Walter K. Fisher, Director of the Hopkins Marine Station of the Stanford University for drawing 2.

muscles. The contractions are of an incomplete tetanic and irregularly periodic type. The contractions can be easily felt as vibrations through the body wall. The sound produced is that of a low growling croaking or grunting noise. The tone pitch and quality produced by the vibrations vary in different specimens. These two factors are characteristic enough so that it is easy to identify the different specimens in the aquarium.

It is difficult to determine the physiological reason for this type of apparatus. One is limited to conclusions from indirect evidence. The fish in the aquarium emitted the sounds under conditions that could be variously interpreted. The largest and most pugnacious specimen of the aquarium, a male 34 cm. in length, used the vocal apparatus under aggres-

Fig. 4. Porichthys notatus. Illustrating the defense reaction often associated with noise production.

sive and defensive conditions. Before the end of the season he would invariably produce the sound when he was punched with a rod. Often he would attack the rod, grasping it with his sharp teeth, at the same time emitting a vigorous and prolonged growling noise. When this specimen aggressively attacked other specimens, they would produce the noise but while swimming away from the aggressor. At other times, when the aquarium was quiet and only the most gentle movements occurred, a more soothing tone of low intensity was used by the various members of the aquarium family as they swam back and forth in each others' proximity. Take it all in all, the behavior of the specimens of Porichthys in this aquarium was not unlike the group behavior of the more familiar land

animals. One could scarcely refrain from the conclusion that these fishes used their noise-producing air-bladders under conditions of colony activity —fright, combat, defense and friendly association.

The closed air-bladders of fishes that swim at different depths have long been known to have a hydrostatic function. Porichthys notatus spawns at tide water and is taken by fishing dredges as deep as 30 or 40 fathoms. The evidence from a study of the gases published elsewhere[2] demonstrates that the gas may be rapidly secreted into the air-bladder. A single helpless gas-bloated specimen found floundering on the surface of Monterey Bay is evidence of a too rapid change of depth whereby the fish lost control of the hydrostatic function of the air-bladder by the gas glands, figure 3. These facts justify the assignment to the air-bladder of Porichthys of two outstanding functions, that of *1*, a hydrostatic organ enabling the fish to control its vertical migrations in the sea; and *2*, a vocal organ functioning in the colony for defense, offense, and colony communication.

A collateral item of especial physiological interest is the great development in the air-bladder of muscles of the voluntary and striated type, yet innervated by the tenth or cranial nerves. These muscles are controlled by voluntary discharges of motor nerve impulses. The whole arrangement is analogous, perhaps the embryological history may prove it homologous, to the innervation and control of the laryngeal muscles in the mammalia.

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PHOSPHORESCENCE OF PORICHTHYS NOTATUS, THE CALIFORNIA SINGING FISH

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Luminescence was observed for the first time in Porichthys notatus in the opening year of the Hopkins Marine Station in 1892. Numerous Porichthys are always observed spawning at tide water along the shores of Monterey Bay in June and July. Living adults are available from the nests, yet phosphorescence was never observed in the free swimming fish. Luminescence was first obtained on the living tissue of the freshly killed specimens. The demonstration was made by the method of electrical stimulation. Excessively strong induction or galvanic currents, measured by the standards ordinarily used in physiological laboratories, were required to induce phosphorescence. Later, phosphorescence was obtained by immersing the fish in ammonia water. The light production stimulated by ammonia was never so brilliant nor so persistent as that obtained by electrical stimulation. It was noted in those early experiments that the development of the phenomenon was physiologically slow. A single uncertain example of mild voluntary phosphorescence was recorded as displayed by an excited aquarium fish in this early series.[1]

During the summer of 1923 the opportunity again occurred for repeating and extending these earlier experiments on breeding fish, the only type available from Monterey at this season of the year. For the purpose of studying the composition of the air-bladder gases several specimens of Porichthys were kept in an aquarium in the research room of the senior

[1] The phosphorescent organs in the toadfish, Porichthys notatus girard. Greene, C. W., Journ. Morph., 1899, xv, 684.

Note. In this early paper diagrams of the distribution and variations of arrangement of the numerous rows of tiny phosphorescent organs is given. Porichthys has a total of over 840 organs. Many of these are small and rudimentary but at least 750 well-developed organs are present in the ventral and ventro-lateral lines. The orientation of the organs is such that they face ventrally or approximately so, those on the sides having their axes quite oblique to the surface. The organs are late in embryological development, become identifiable only when the embryo has reached a length of 10 mm. and are adult-like only when the young become free swimming at 25 to 30 mm. length. The paper gives a series of drawings to show the stages of development. Detailed adult histological structure is also given and figured.

author from late June till August 29. Their activities were observed incidentally to other work daily during that season. This revealed a number of interesting characteristics of the behavior of the species.

Phosphorescence at best is only a faint light. It can be observed best with night adapted eyes in the dark room. Periodical efforts to observe the phenomenon of phosphorescence in normal specimens of Porichthys in the darkened laboratory without disturbing the fish in the aquarium were not successful in this artificial environment. However, on two favorable occasions phosphorescence was observed in aquarium fish when violently agitated by the method of prodding with a wooden rod. The light was not strikingly intense, and the difficulty of securing its emission was greater than one would expect on the theory of nerve controlled organs.

By electrical stimulation with induction shocks of comparatively strong intensity applied through the body of the living fish, it is easy to stimulate the phosphorescent organs to emit light. This confirmation of observations in 1892 was made on many specimens, both from Monterey Bay and San Pedro Harbor. The maximum intensity of the light is enough in the dark room to illuminate the features of an observer sufficient for identification, when the fish is held 10 to 12 inches in front of the face. The light is easily visible anywhere in the dark room. Lecture demonstrations were made to groups of 10 or 12 by this method. Indeed, when the whole series of over 800 organs are aglow at one time it is a very unique biological display.

When Porichthys is stimulated by tetanic inductions for two or three seconds the phosphorescent glow begins but only after a latency of from 8 to 10 seconds. It increases in intensity to a maximum at about 15 seconds, then slowly fades to disappear in from 20 to 25 seconds. If stimulated for a longer time the phosphorescence continues for a correspondingly longer period. The latency was longer than that usually observed in the most sluggish of smooth muscle tissue, although smooth muscle contractions last longer in response to stimuli of short duration than does the duration of the light.

The length of the latent period and the general character of the wave of development and waning of phosphorescence are suggestive of a chemically controlled gland rather than of one under nerve control. In fact the senior writer earlier failed to observe any definite nerve supply in numerous histological preparations of adult and semi-adult glands. Individual nerve fibers to the phosphorescent organs were observed, but even these were not so prominent in the glandular part of the organ as in the lens. The gland is well supplied with blood vessels and capillaries as shown by Greene in figure 5 of plate XXXIXa of the paper on development.

Fig. 1. Porichthys notatus photograph to show the relative size and distribution of the phosphorescent organs visible from the ventral view. Most of the phosphorescent organs shown on the photograph have been retouched to strengthen their contrast. An average of 308 organs on each side, namely, a total of 616, are visible from the ventral view forming in truth the fish's "own white way." In the photograph certain organs are covered by the ventral and anal fins. There are well developed lines on the sides and lines with numerous rudimentary organs on the dorsal surface of the head and trunk that bring the grand total to well over 840 organs. The exact number varies rather widely in individual specimens.

Opportunity did not offer during the season at Hopkins Marine Station for tests of hormone control save for a single brilliant exception. Adrenalin hydrochloride was tested at the time of closing the summer's work on August 29, 1923. A vigorous medium sized male Porichthys was observed in the dark room. After dark adapting the eyes of the observers, electrical stimulation was used which demonstrated that this particular fish was capable of phosphorescence. Vigorous effort to excite the fish and thereby secure voluntary display of phosphorescence failed. These stimuli were vigorous and strong and produced a copious increase in the secretion of mucous by the glands of the skin.

At this point 0.25 cc. adrenalin hydrochloride was injected subcutaneously into the belly wall of the right side immediately under the prominent gastric line of phosphorescent organs. After a rather pronounced latent period, two or more spots above the area of injection began to show luminescence. These organs were not contiguous but well separated in the row. A few seconds later (the time could not be accurately measured in the dark room) individual organs in adjacent lines began to glow faintly, then several more organs in the posterior segment of the gastric line under which the injection occurred became visible. From this time on individual organs on both sides of the abdomen, of the anal lines, and mandibular lines, that is, in regions distant from the point of injection but still in irregular areas, became visible. It was noteworthy that no true sequence or order or uniformity in the degree of light intensity occurred at first. In the course of approximately 10 minutes, the entire phosphorescent organ line system became visible and of uniform brightness. The light was bright enough to produce a distinct reflection of light from the surface of the wall and from the bottom of the glass aquarium, also from the surface of the water. The outlines of one's hand and fingers were easily distinguishable but the intensity did not in this experiment become great enough to illuminate the features of observers near the aquarium sufficient for identification.

The illumination continued uninterrupted and with no observable difference in intensity for over one hour. The glow was still continuing strong when the observations had to be discontinued. In this regard the stimulation by adrenalin differed markedly from the electrical stimulations. Additional adrenalin was injected into the abdominal cavity after about 40 minutes. However, it did not induce any greater intensity of illumination and of course one can not assert what influence the second injection had on the total prolongation of luminescence.

In comment on these experiments, the outstanding facts are: 1, the time differential in the development of phosphorescence among individual organs after the injection of the hormone; 2, the long latent period, not only in this chemical test, but in electrical tests; 3, adrenalin is known

as a specific stimulator of the myoneural junctions of the mammalian thoracic sympathetics, yet this can scarcely be the mechanism of action in Porichthys in view of the morphological failure to demonstrate nerves to the phosphorescent organs. The reaction would seem to indicate a direct hormone action on the phosphorescent gland cells themselves, or possibly on the metabolic products of these cells.

The hormone tests have been repeated by the junior author at intervals during the winter at the California Fish and Game Laboratory at East San Pedro, California.[2] Such specimens of Porichthys as became available and could be revived in the laboratory after collection by the sardine fishermen were put to the chemical tests indicated in table 1. Demonstrations of the power of active phosphorescence were made by the electrical stimulation method preliminary to chemical treatment. Adrenalin injections were made on 7 specimens. Every one of these tests resulted in the production of phosphorescence. Each was characterized by continuous light production for a prolonged interval. The exact duration of the phenomenon was not determined in each instance. But observation was continued long enough to indicate a similarity of reaction to that of the initial observation on the specimen from Monterey Bay. Sex was not always recorded, but both males and females were demonstrated. It will be noted that the fishes were collected in February, March and April, all non-spawning months. This fact effectively answers the question raised by Greene's earlier paper that active phosphorescence in Porichthys might be only a breeding seasonal phenomenon. It is not such.

We are assuming for the present that in Porichthys the phosphorescent organs are hormone controlled. The assumption is based 1, on the nature of the structure of the glandular part of the organ; 2, on the absence of demonstrated nerve supply; 3, the rich vascular supply to the gland; 4, the slow rate of development of phosphorescence by any method induced; and finally 5, the prolonged duration of luminescence under hormone stimulation. The duration is literally hours without interruption. No conception of nerve control of either a glandular or a muscular organ fits this outstanding fact.

Adrenalin is recognized as a specific thoracic autonomic stimulator of nerve endings. But there is increasing evidence that this substance is not without direct influence on muscle, especially in denervated organs.[3] We are constrained to add gland to the tissues that may be directly stimu-

[2] We are particularly indebted to the Director of the Laboratory, Professor Thompson, not only for personal courtesies but for numerous facilities and apparatus in establishing aquaria and aerating devices in a laboratory of general equipment only.

[3] Meltzer and Auer: This Journal, 1904, xi, 40. See also Cannon, This Journal, 1919, I, 399.

TABLE 1

Reactions of the phosphorescent organs of Porichthys notatus upon single injections into the abdominal wall or the peritoneal cavity of adrenalin hydrochloride (P. D. Co.) numbers 1 to 7, of pituitrin obstetric 00 (P. D. Co.), or insulin (Lilly)

NUMBER	DATE	DRUG AND AMOUNT	FIRST LIGHT		MAXIMAL LIGHT		TOTAL DURATION OBSERVED	
			minutes	*seconds*	*minutes*	*seconds*	*hours*	*minutes*
1	February 12	Adrenalin 0.5 cc. 0.01 per cent						
2	February 12	Adrenalin 0.5 cc. 0.1 per cent	1	50	2	00	1	40
3	February 15	Adrenalin 1.0 cc. 0.1 per cent	0	45			0	20
4	February 20	Adrenalin 0.3 cc. 0.1 per cent	0	30	1	50		
5	March 9	Adrenalin 0.1 cc. 0.1 per cent	0	37	1	25	*	
6	March 9	Adrenalin 0.2 cc. 0.1 per cent						
7	March 25	Adrenalin 1.0 cc. 0.01 per cent	1	15	5	15	2	20
8	March 31	Pituitrin obstetric 00, 0.5 cc.	0	45	1	45	0	39
9	April 20	Insulin 0.5 cc.	None		None		None	

* Not observed.

Notes on the experiments of table 1:

Experiment 1. This first fish was set aside as a failure after injection but later it was found that faint light appeared in the rows of organs and continued visible for several minutes.

Experiment 2. The light first appeared in the rows of the anterior parts of the body in front of the rubber band which was used to bind the fish to the holder. When this rubber band was lifted the lights of the tail came out promptly as if the circulation had been blocked. The organs were luminous when observed after 1 hour, 40 minutes, but had ceased to glow at the next observation, 2 hours, 40 minutes.

Experiment 3. This fish was found in death struggle. After 3 minutes it was injected with adrenalin. The lights appeared promptly but were dimmer than in no. 2. Still luminous after 20 minutes.

Experiment 4. The first organs to become luminous were scattered, but all were shining after 47 seconds. The total duration was not set down but notes state that "it was a matter of hours".

Experiment 5. The organs shone out irregularly in the rows at 37 seconds but all lights were out in 55 seconds and brilliant 30 seconds later. Duration of lights not determined.

Experiment 6. In number 6 the syringe was emptied accidentally in the dark and only an unknown small amount of adrenalin was actually injected. A few scattered dim lights appeared, chiefly near the point of skin puncture.

Experiment 7. This dose compares with the first test injection in no. 1. All organs lighted slowly and uniformly, in contrast with the reaction to stronger solutions. On handling the fish after 2 hours, 20 minutes, the lights which had become very faint were perceptibly brighter following struggling. No later examination was made.

Experiment 8. The organs became luminous to pituitrin injection very evenly over the rows, but the lights at the brightest were dim compared with the adrenalin series.

Experiment 9. Insulin injection produced no light reaction in the phosphorescent organs within two hours' observation.

lated, in this instance the somewhat special type of gland, phosphorescent cells.

Whatever the process going on in the phosphorescent cells it is questionable whether sugar metabolism, at least that factor influenced by insulin, plays any part. This was to be expected. But the fact that pituitary extract, pituitrin, does so stimulate is unexpected. We present only the interesting fact as a preliminary announcement and await our further experiments to clarify the subject.

One may assume that the chemical mechanism in Porichthys is comparable to that in the fireflies, glow worms and numerous marine invertebrates in which Dubois, Harvey, and others have demonstrated definite chemical entities, luciferin and luciferase. The organs in Porichthys, while very numerous, are exceedingly small. The mass of the organs in the skin bears a very low ratio to the total skin. Nevertheless, it is hoped that the chemical agents responsible for the production of phosphorescence in Porichthys may yet be isolated.

SUMMARY

1. Confirmation and extensions are made of the observations of Greene that the organs of the shore fish, Porichthys notatus, are truly phosphorescent organs. That the fish can under intense excitement be made to produce light voluntarily is confirmed.

2 Phosphorescence of short duration but of a brilliant display can easily be induced by strong general stimulation.

3. Under injections of the hormone of the supra renal gland the phosphorescent organs become brilliantly luminous and remain so for hours after a single injection.

4. It is suggested that these organs are hormone controlled rather than under nervous coördination.

STUDIES UPON THE VASCULAR AND CAPILLARY PHENOMENA AND SUPPOSED AXON REFLEXES CONCERNED IN THE DEVELOPMENT OF EDEMA IN MUSTARD OIL CONJUNCTIVITIS, TOGETHER WITH THE EFFECTS OF VASODILATOR DRUGS, LOCAL ANESTHETICS AND VITAL STAINS[1]

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The ever-interesting problem of edema, with the many experiments and theories which have been devised to throw light upon the mechanism which brings it about, has been so thoroughly reviewed in Leo Loeb's recent monograph that no new discussion of the literature is necessary, but the very completeness of this literary review brings to light a number of points which need the test of further experimental investigation.

The present study concerns itself with the elucidation of three points:

1. The importance of circulatory versus colloidal factors in the development of edema

2. The importance of the rôle of vasoconstriction and vasodilatation, rather than of the intactness of the sensory fibers concerned in the axone reflexes

3. The usefulness of vital stains for observing the condition of the capillary walls in the absence of definite edema.

The older theory of Ludwig, Cohnheim and Starling that edema develops as the result of filtration through the walls of injured capillaries, was rudely set aside by Martin Fischer, who claims that the only important factor in edema is increased imbibition of water by the colloids of the injured tissue due to the presence of acids which are formed as a result of the injury.

If Fischer's theory were correct, the fluid present in inflammatory edema should be more acid than normal tissues and tissues subjected to inflammation should imbibe water and become edematous when immersed in blood serum outside of the body.

If the older filtration theory is correct, the edema fluid should be increased under all conditions in which the capillary filtration pressure is increased

[1] The experiments reported in this investigation were aided in part by a Grant from the Research Fund of the Graduate School of the University of Minnesota.

and should be diminished under all conditions in which the capillary pressure is diminished.

The attempt to discard mechanical factors and to ascribe the development of edema to increased absorption of water by the colloids of the connective tissues was made by Martin Fischer in 1908. Fischer demonstrated that the tissues of the excised kidney took up more water in the presence of dilute acids than they did in neutral solutions.

Fischer extended to the excised kidneys the studies which Ranke and Jacques Loeb had made upon striated muscle tissue, namely, that in the presence of dilute acids these tissues took up more water from the surrounding media than they did in the absence of these acids. Since Araki had shown that lactic and other acids were formed in tissues during asphyxia, Fischer reasoned that the presence of these acids in injured cells caused them to take up more water than normally and thereby bring about swelling of the cells which he regards as synonymous with edema. Fischer's theory differed from Loeb's in one respect, viz., that Loeb regarded the swelling as a matter of pure osmosis, while Fischer regarded it as a phenomenon of increased hydration of the colloids similar to the swelling of gelatin or fibrin under similar conditions. Cannon has shown that brain tissue, after mechanical injury, swells in the same way, and MacNider has demonstrated the same for kidney cells in nephrosis from metallic poisons and general anesthetics. Their studies may be regarded as confirmation of Fischer's theories.

However Fischer's theories may apply to the swelling of the parenchyma cells in injured organs, as is generally encountered in cloudy swelling, he makes no differentiation between the imbibition of water by parenchyma cells and the accumulation of edema fluid in tissue spaces in the ordinary interstitial edema of transudations and inflammatory exudates.

These two processes are by no means necessarily homologous, and much evidence can be advanced to show that the development of interstitial edema is much more dependent upon circulatory factors than upon imbibition by the colloids.

The edema which develops in the subconjunctival tissue of the rabbit's eyelid after instillation of mustard oil furnishes an ideal site for study since one lid can be cut out and its cross section examined at any stage, while the lid of the other eye can be retained as a control, or can be treated with mustard oil and cut out before the test experiment is begun.

In 1916 I was able to show that the edema fluid which collects in the subconjunctival tissue after the instillation of mustard oil into the conjunctival sac exists in the interstitial tissue spaces and that, after the tissue is cut, it is easily expressed by very slight mechanical pressure. Moreover, if the eyelid is excised within five minutes after the application of mustard oil, but before the edema has begun to develop, the lid does not swell and no

edema develops if the lid is immersed in 0.9 per cent NaCl solution, Ringer-Locke solution or rabbit's serum, showing that the development of edema is not due to imbibition of fluid by the tissues.

It was also possible to demonstrate that there is no appreciable accumulation of acid in the edema fluid. When the reaction of this fluid was tested with phenolsulphonephthalein it showed the same tint as the blood of the animal (about pH 7.35), and more recently I have found that it also gives the same reaction as the serum, a definite reddish color, with powdered cresol red.

This method is, of course, a rough one and gives results slightly higher than have been obtained, since my experiments were performed, by Schade, Neukirch and Halpert. These observers tested the hydrogen ion concentration directly with the H cell, using Pt electrodes introduced directly into a single drop of the fluid to be tested. They found that the pH of the serous exudates which they examined ranged from 7.00 to 7.10 while that of the transudates ranged from 7.15 to 7.20. Boots and Cullen, however, found that the pH of edema fluid in acute and chronic arthritis ranged from 7.2 to 7.4, which is in close accord with my color tests upon the fluid of mustard oil edema.

The edema is therefore not associated with either local acidosis or imbibition of water by the tissue colloids.

A study of mustard oil edema furnished, however, excellent proof for the theory of Ludwig, Cohnheim and Starling that edema is formed by the filtration through the walls of injured capillaries; and that its amount, other things being equal, should be roughly proportional to the difference between pressure in the capillaries and that in the surrounding interstitial tissue.

In our experiments, which were performed in 1917, we have confirmed the work of Bardy who found that instillation of epinephrin (adrenalin) into the conjunctival sac inhibited the development of edema on the treated side.

The suppression of edema by epinephrin is, however, transitory, and after two hours when the vasoconstriction has passed off, edema develops normally.

This is shown in the following typical experiments, which were repeated upon several series of animals.

TABLE 1

Effect of instillation of three drops of adrenalin hydrochloride 1:1000 into one conjunctival sac

TREATED LIDS (EXCISED 1 HOUR LATER)	CONTROL LID
−	++
−	++
−	++
−	++
Very slight	++

+ = definite edema; ++ = marked edema; +++ = very intense edema; − = edema absent.

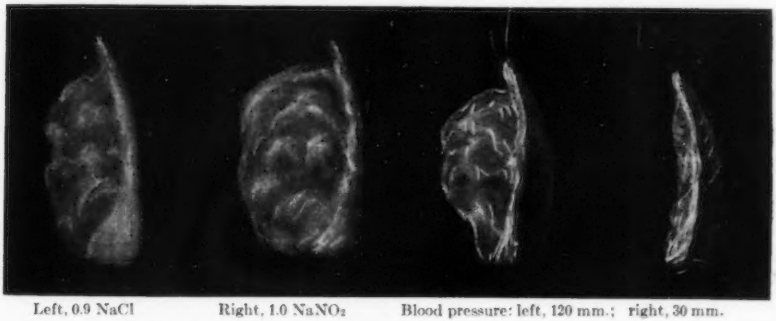
TABLE 2

	JULY 22, 1924					JULY 23, 1924
	9:55 a.m	11:40 a.m.	2:00 p.m.	4:30	6:00	11:00 a.m.
	Brown rabbit					
Left eye....	2 drops mustard oil + 2 drops adrenalin 1:1000	−	+	+ Almost as much as in right eye	++ Same as right eye	++
Right eye...	2 drops mustard oil only	++	++	++	++	++
	White rabbit					
Left eye....	2 drops mustard oil + 2 drops adrenalin 1:1000	Very slight edema of upper lid, none of lower	Slight edema in both lids	+ Almost as much as in right eye	++ Same as right eye	++
Right eye...	2 drops mustard oil only	++	++	++	++	++

This proves that the epinephrin does not protect the capillary walls from injury but merely inhibits the edema during the period when the filtration pressure in the capillaries is kept down by constriction of the arterioles. Further proof for the immediate injury of the capillaries is given below in the experiments upon vital staining (fig. 11).

Bardy also obtained with instillation of nicotin, suppression of the edema similar to that brought about by adrenalin. He also found, as Samuels had found for croton oil edema in the rabbit's ear, that if one carotid artery was ligated, the edema of the conjunctiva was greatly inhibited on the operated side. Under these circumstances we found that the pressure in the cephalic end of the cut carotid was less than 40 mm. Hg, while that at the proximal end was 100 to 120 mm. Hg. On the other hand I have found that pure vasodilatation increases edema only when it also increases the capillary pressure. For example, 1 per cent sodium nitrite locally increases the edema (fig. 1), whereas 1 per cent sodium nitrite

given intravenously until the general arterial pressure remains at 20 to 40 mm. Hg greatly diminishes the edema (figs. 2 and 3). The same is true of Witte's pepton. Still further, any condition which greatly lowers the general blood pressure inhibits the development of edema.



Left, 0.9 NaCl Right, 1.0 $NaNO_2$

Fig. 1. Effect of vasodilatation from 1 per cent $NaNO_2$ instilled into conjunctival sac on the development of edema.

Blood pressure: left, 120 mm.; right, 30 mm.

Fig. 2. Effect of a spontaneous fall in blood pressure on the development of edema.

TABLE 3

Sodium nitrite instilled into conjunctival sac followed by mustard oil

	0.5 PER CENT $NaNO_2$	CONTROL—0.9 PER CENT NaCl
Cat	++	++
	1.0 PER CENT $NaNO_2$ (REPEATEDLY)	CONTROL
Cat	+	Slight
Cat	++	+
Rabbit	++	+
Rabbit	++	+
Rabbit	++	+
Rabbit	++	++
Rabbit	++	++
Rabbit	+++	++
Rabbit	+++	++
Rabbit	+++	++

On the other hand, G. Spiess has claimed that local anesthesia partly or completely inhibits the development of inflammatory edema, and Ninian Bruce has shown that the application of 10 per cent cocaine or 10 per cent alypin to the conjunctiva prevents the development of the edema. Bruce

TABLE 4

Effect of sodium nitrite intravenously in the cat

	BLOOD PRESSURE	EDEMA
	mm. Hg	
Control (before injection of $NaNO_2$ but 1 hour after mustard oil instillation)	120	++
After injection of $NaNO_2$ 1 hour after mustard oil instillation	30	Very slight
Normal	120	++
After 2 cc. 2 per cent $NaNO_2$ intravenously	45	Very slight
Control	120	++
After 2 cc. 2 per cent $NaNO_2$ intravenously	20	−
Control	100	++
After 1.5 cc. $NaNO_2$ intravenously	40 falling to 20, dead	Trace
Control	120	++
After 1.5 cc. $NaNO_2$	30–21	Very slight

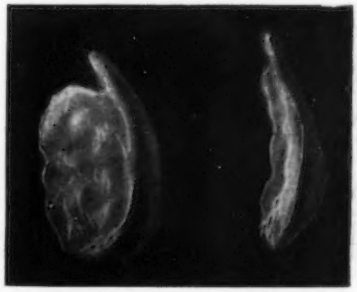
TABLE 5

Effect of 1 per cent Witte's pepton intravenously in rabbits

The control lid was treated with mustard oil and removed one hour later. After this the Witte's pepton solution was injected intravenously in doses not sufficient to produce shock, mustard oil was instilled into the remaining conjunctival sac and the latter was removed one hour thereafter.

CONTROL	AFTER PEPTON
++	+++
Trace	+
+	++
Trace	++
+	++
+	++
++	++
+	++
+	++
+	++
++	++
+	+
+	++
+	++
++	++
+	+

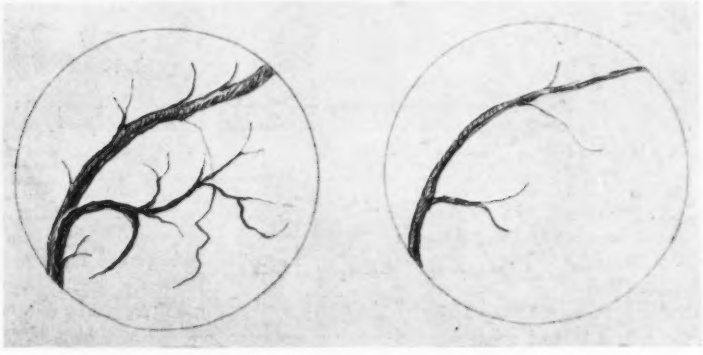
found that if the ophthalmic nerves are cut and mustard oil applied to the conjunctiva, edema still develops, but if these nerves are allowed to develop completely, edema does not develop. Bardy has confirmed Bruce's findings. Bruce regards this as absolute proof that the development of edema is dependent upon the intactness of the axon-reflex whose existence has been presupposed by Langley and Bayliss, viz., that the sensory nerves have a Y-shaped branching in which one arm of the Y goes to a sensory ending and one arm goes to a vasodilator nerve ending. Krogh and his pupils accept Bruce's theory. Since all our results seemed to point to the importance of vasodilatation as a factor in the deve opment of edema, and since cocaine and alypin themselves exert a marked vasoconstrictor action (figs. 4, 5), it seemed important to repeat Bruce's experiments using some of the newer local anesthetics which either do not affect the blood vessels or actually dilate them.



Left, before $NaNO_2$ — Right, after 2 cc. 1 per cent $NaNO_2$ intravenously

Blood pressure 80-100 mm. — Blood pressure 20-40 mm.

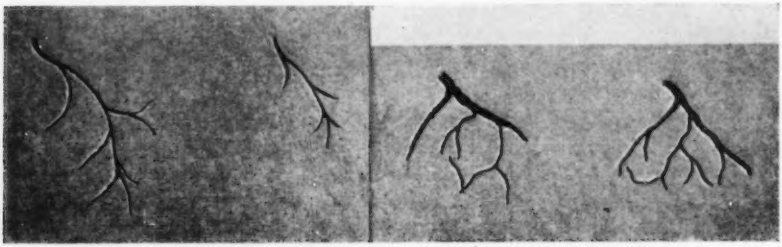
Fig. 3. Effect of lowering blood pressure with $NaNO_2$ on the development of edema.



4.30 p. m., normal — 4.43 p. m., same vessels after instillation of 10 per cent cocaine

Fig. 4. Effect of cocaine on the caliber of the small arteries and capillaries of the rabbit's lid. Magnification 90.

We used 4 per cent cocaine; 4 per cent procaine, which has little effect on the blood vessels; 4 per cent saligenin, which has a definite vasodilator action (fig. 6), and 4 per cent butyn, which has a somewhat greater local anesthetic power than cocaine but has some vasodilator action (fig. 7).



A, 4:40 p.m., normal conjunctival tissue pink

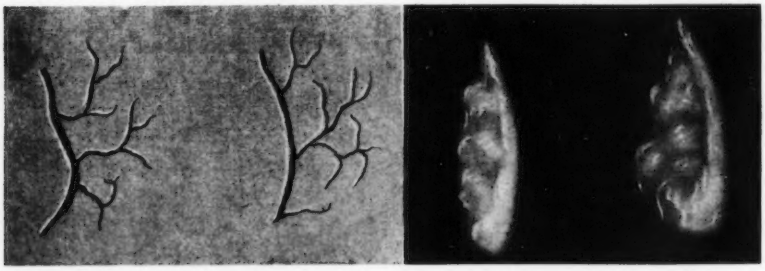
B, 4:48 p.m., after 10 per cent alypin, at 4:45 conjunctival tissue white

Fig. 5. Effect of alypin on the caliber of the small arteries and capillaries of the rabbit's lid.

A, 3:45 p.m., normal

B, 3:43 p.m., after 4 per cent saligenin at 3:41

Fig. 6 Effect of saligenin on the caliber of the small arteries and capillaries of the rabbit's lid.



A, 4:04 p m , normal

B, 4:12 p.m., after 2 per cent butyn at 4:08

Fig. 7. Effect of butyn on the caliber of the small arteries and capillaries of the rabbit's lid.

After 10 per cent cocaine followed by mustard oil

Control mustard oil only

Fig. 8. Effect of the instillation of 10 per cent cocaine hydrochloride upon the development of edema.

The mustard oil was not applied until the conjunctiva was definitely anesthetic when tested with a piece of fine copper wire. The animal did not wink, scream or show signs of pain when the mustard oil was applied to the anesthetized eye, but did so when it was applied to the control eye. Our results are shown in the table 6.

However, even 10 per cent cocaine gives uncertain results. Thus in a series of four rabbits which received three instillations of 10 per cent cocaine within one half-hour, every one developed edema within an hour

though it developed a little more slowly than in the controls, and in one control the cocainized eye showed about one-half the edema that was present in the control (fig. 8). Similar results were obtained with 10 per cent alypin.

This variability in the reaction in itself should lead us to be somewhat conservative in accepting an hypothesis like that of the axon-reflex reaction

TABLE 6

Effects of instillation of local anesthetics into the conjunctival sac of the rabbit. Lids removed one hour after the application of the mustard oil. Anesth. = lid of the anesthetized eye

Three drops of the anesthetic were injected into the conjunctival sac of the anesthetized eye, and this was repeated twice at 15-minute intervals. The lids and sclerae were found to be anesthetized when touched with a copper wire; and the animals did not scream when the mustard oil was injected into the treated eye, as they did with the untreated.

4 PER CENT COCAINE		4 PER CENT PROCAIN		4 PER CENT SALIGENIN		2 PER CENT BUTYN		10 PER CENT COCAINE	
Anesthetized	Control	Anesthetized	Control	Anesthetized	Control	Anesthetized	Control	Anesthetized	Control
−	++	Slight	++	++	+	++	++	++	++
++	++	+	+	++	++	++	++	++	++
++	++	++	++	+	++	++	++	++	++
		++	++			++	++	+	++
								++	++
								++	++
								+	++
								++	++
								++	++

\+ = definite edema.
++ = very intense edema.

without further proof, and some of this skepticism seems to be shared by Langley in his article in the October 22, 1923, number of the Journal of Physiology.

It is easy to demonstrate, however, that the absence of edema does not indicate that the capillary walls have been guarded from injury. For this purpose one can use intravenous injections of trypanblue (figs. 9 and 10).

Evans, Bowman, Winternitz and Hirschfelder found that this dye stains tubercles in experimental tuberculosis, and Winternitz and Hirschfelder demonstrated that it stains the consolidated area of lung inexperimental lobar pneumonia selectively. When I injected 10 cc. 1 per cent trypanblue (in 1.5 per cent salt solution—to avoid hydremia) into the ears of a rabbit whose left eye was treated with adrenalin before applying the

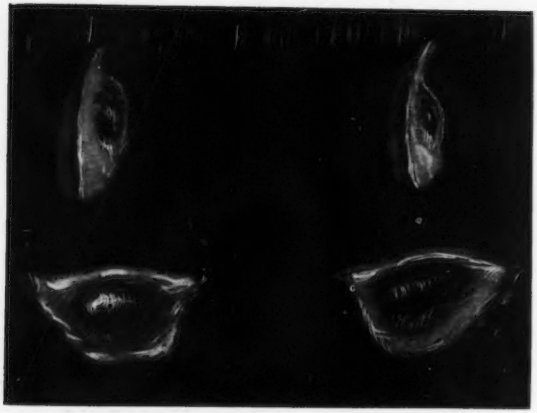


Fig. 9. Edema and deep blue staining in eyelids after 10 per cent cocaine (right eye) and trypanblue. Ten per cent cocaine instilled into right conjunctival sac at 4:30 p.m. and 5 p.m.; 2 drops of mustard oil into both eyes at 4:55 p.m. One per cent trypanblue in 1.5 per cent NaCl solution injected intravenously at 5:15 p.m. Both lids were removed at 5:50 p.m. showing edema in both eyes, less in cocainied eye, but deep blue staining in both.

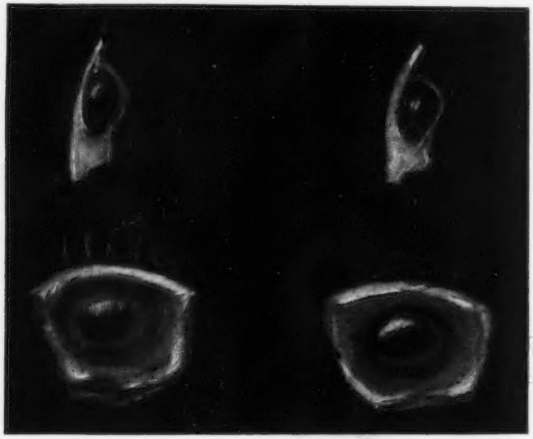


Fig. 10. Edema and trypanblue staining after alypin and mustard oil into right eye, and trypanblue intravenously.

mustard oil, the dye stained the treated area more deeply than normal even though no edema had developed within an hour before the dye was injected. Cocainized lids showed the same phenomenon (fig. 11).

This can be taken as evidence that these agents do not protect the capillary walls, even when they reduce the filtration pressure between the capillaries and the extra-capillary spaces.

Fig. 11. Cocaine and adrenalin in left eye (no edema); trypanblue intravenously. Vital staining shows injury of capillaries in spite of absence of edema.

Another viewpoint has been advanced by Chiari and Januschke who found that preliminary subcutaneous injections of calcium chloride prevent the development of edema, and they regard this as proof of the conception that the calcium salt alters the physicochemical properties of the endothelial walls and perhaps also the supposed power of imbibition of the surrounding tissues. Their observations have been confirmed by some other observers.

On the other hand, this remedy also is uncertain in its action as is shown by the following typical experiments: Three rabbits received 4 cc. 5 per cent $CaCl_2$ subcutaneously at 2:35 p.m., 3:35 p.m. and 4:40 p.m. Immediately after the third injection 2 drops of mustard oil were instilled into the left conjunctival sac of each. All three developed the typical edema within half an hour. Trypanblue intravenously stained the edematous area very deeply.

2:35 p.m., normal

3:38 p.m. after 4 cc. 5 per cent $CaCl_2$ subcutaneously at 2:30 and 3:30 p.m.

4:40 p.m.

Fig. 12. Effect of $CaCl_2$ subcutaneously on the arterioles and capillaries of the rabbit's conjunctiva.

It is probably significant that before the application of mustard oil, the conjunctival vessels were dilated, and in one which was watched under the capillary microscope, dilatation of the smaller blood vessels and arteries was observed (fig. 12). It seems not unlikely that the inhibition of

edema may occur in animals in which the calcium chloride induces vasoconstriction instead of vasodilatation, but this point has not been proved.

In the performance of many of these experiments I was assisted by Dr. M. Nathanson and Messrs. R. Hultkrans, J. May and H. Weber, whose hearty coöperation I gratefully acknowledge.

SUMMARY AND CONCLUSIONS

1. In the inflammatory edema in mustard oil conjunctivitis, the edema fluid is about neutral to cresol red (pH log -7.3 to -7.5). There is therefore no sign of local acidosis.

2. When excised after treatment with mustard oil, the lid does not swell in rabbits' serum, isotonic salt solution or in Ringer-Locke's solution. The edema is therefore not produced by imbibition of water by the colloids.

3. The edema is decreased by local vasoconstrictors, by ligation of a carotid artery, and by epinephrin intravenously; and it is increased by local vasodilatation, unless the general blood pressure has fallen considerably.

4. Even when the edema has been inhibited, injury to the capillary wall can be demonstrated by the selective staining which occurs when trypanblue is injected intravenously.

5. Ten per cent cocaine and 10 per cent alypin when applied to the rabbit's conjunctiva produce marked vasoconstriction. Four per cent cocaine does so to a much less marked degree. Four per cent procaine, 2 per cent butyn and 4 per cent saligenin which produce complete anesthesia of the conjunctiva do not cause this vasoconstriction.

6. In spite of the intense anesthesia produced, and of some vasoconstriction, the cocaine and alypin do not inhibit the development of edema from mustard oil with any reliable regularity; and saligenin, procaine and butyn do not do so at all.

7. It seems probable therefore that the inhibiting action of cocaine and alypin is due to their vasoconstrictor action, rather than to an axon-reflex reaction.

8. Calcium chloride does not always inhibit the development of mustard as claimed by Chiari and Januschke. In some, at least, of the animals in which edema is not inhibited, vasodilatation takes place after calcium chloride administration.

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EFFECT OF INSULIN IN EXPERIMENTAL INTOXICATION WITH ALCOHOL AND ACETONE

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Since Banting and his collaborators have shown that injections of insulin cause an increased oxidation of glucose, and that injections of glucose will cut short the convulsions produced by injections of insulin, it seemed of interest to test the effect of insulin upon the course of intoxications with alcohol and acetone.

Rabbits were injected with 50 per cent alcohol by stomach tube. After somnolence or coma had been induced, animals were given iletin (Eli Lilly & Co.) as shown in the tables below.

Experiments April 8, 1924

	EXPERIMENT 1—RABBIT, BLACK, WEIGHT 2700 GRAMS	EXPERIMENT 2—RABBIT, TAN, WEIGHT 2400 GRAMS
1:10 p.m.	5 cc. alcohol	5 cc. alcohol
2:30 p.m.	5 cc. alcohol	5 cc. alcohol
3:15 p.m.	5 cc. alcohol	5 cc. alcohol
3:30 p.m.	Severe prostration	Severely prostrated
3:50 p.m.	4 units insulin injected	4 units insulin injected
5:50 p.m.	Still in coma	Still in coma
Next morning	All right	Dead

Experiment April 29, 1924

TIME	RABBIT 3, 2700 GRAMS	RABBIT 4, 2300 GRAMS
1:00 p.m.	12 cc. alcohol (50%)	10.5 cc. alcohol (50%)
2:36 p.m.	Coma	Coma
2:40 p.m.	4 units insulin	4 units insulin
3:20 p.m.	Raised head	Coma
3:30 p.m.	Runs about	Coma
4:35 p.m.	Normal	Raises head

Experiment May 15, 1924

TIME	RABBIT, 1125 GRAMS	RABBIT, 1100 GRAMS	RABBIT, 1025 GRAMS	RABBIT, 1050 GRAMS
2:20 p.m.	10 cc. alcohol (50%)	9.5 cc. alcohol (50%)	9 cc. alcohol (50%)	9 cc. alcohol (50%)
3:15 p.m.	No signs of prostration			
3:50 p.m.	5 cc. alcohol (50%)	5 cc. alcohol (50%)	5 cc alcohol (50%)	5 cc. alcohol (50%)
4:05 p.m.	All severely intoxicated (coma)			
4:10 p.m.	4 units insulin	4 units insulin	4 units insulin	No insulin
4:40 p.m.	Held head up	Coma	Coma	Coma
4:55 p.m.	Dead	Coma	Coma	Coma
5:30 p.m.		Coma	Coma	Raised head
5:50 p.m.		Coma	Coma	Some activity

Experiment May 1, 1924

TIME	RABBIT, 2600 GRAMS	RABBIT, 2325 GRAMS
11:25 a.m.	12 cc. alcohol (50%)	11 cc. alcohol (50%)
1:00 p.m.	Coma	Coma
1:10 p.m.	4 units insulin	No insulin
2:40 p.m.	Coming out	Coma
2:50 p.m.	Runs about	Raises head
3:10 p.m.	Runs about	Runs about

Experiment May 28, 1924

TIME	RABBIT, 1150 GRAMS	RABBIT, 1600 GRAMS	RABBIT, 1100 GRAMS	RABBIT, 1200 GRAMS	RABBIT, 1450 GRAMS
1:20 p.m.	11 cc.	14 cc.	10.5 cc.	11 cc.	12 cc.
2:10 p.m.	Coma	Coma	Coma	Coma	Coma
2:15 p.m.	No insulin	No insulin	4 units insulin	4 units insulin	4 units insulin
2:30 p.m.	No insulin	No insulin	4 units insulin	4 units insulin	4 units insulin
3:00 p.m.	Coma	Coma	Lifts head	Coma	Lifts head
4:45 p.m.	Raises head	Holds up head	Coma	Coma	Holds up head
5:30 p.m.	Sits up	Sits up	Coma	Sits up	Still intoxicated

Experiment May 29, 1924

TIME	RABBIT, 1250 GRAMS	RABBIT, 1235 GRAMS	RABBIT, 1235 GRAMS	RABBIT, 1230 GRAMS
2:00 p.m.	11 cc. alcohol (50%)	11 cc. alcohol (50%)	11 cc. alcohol (50%)	11 cc. alcohol (50%)
3:30 p.m.	Prostrated	Prostrated	Prostrated	Very somnolent
3:35 p.m.	Prostrated	Prostrated	Prostrated	Coma
3:38 p.m.	4 units insulin	4 units insulin	4 units insulin	No insulin
4:20 p.m.	Holds head up	Coma	Coma	Holds head up
5:00 p.m.	Holds head up	Coma	Coma	Holds head up
5:40 p.m.	Runs about	Sits up	Sits up	Runs about

As seen in the above-mentioned experiments, there were no striking differences between the animals that received insulin and those which did not. This would seem to indicate that insulin does not materially alter the course of intoxication with ethyl alcohol or acetone. A couple of animals which had been given insulin subcutaneously until convulsions were produced, received doses of 20 per cent alcohol intravenously corresponding to the amounts used in the above mentioned experiments. These doses did not influence the course of insulin convulsions.

In another series of experiments, rabbits were given acetone by stomach tube in amounts sufficient to produce coma. After this had set in, insulin was administered subcutaneously as shown in the tables below.

Experiment May 22, 1924

TIME	RABBIT, 1250 GRAMS	RABBIT, 1230 GRAMS	RABBIT, 1230 GRAMS	RABBIT, 1250 GRAMS
3:10 p.m.	8 cc. acetone	8 cc. acetone	8 cc. acetone	8 cc. acetone
3:20 p.m.	Coma	Coma	Coma	Coma
3:33 p.m.	4 units insulin	4 units insulin	Control	4 units insulin
3:44 p.m.	Sat up	Coma	Coma	Coma
4:15 p.m.	Sat up	Coma	Coma	Sat up
4:45 p.m.	Moves about	Very drunk	Head up	Moves about
5:00 p.m.	Active	Very drunk		Active
Next morning			Dead	Dead

Experiment May 28, 1924

TIME	RABBIT, 2200 GRAMS	RABBIT,[1] 2200 GRAMS	RABBIT, 1500 GRAMS	RABBIT, 3300 GRAMS	RABBIT, 3700 GRAMS
1:50 p.m.	10 cc. acetone	10 cc. acetone	6 cc. acetone	12 cc. acetone	12 cc. acetone
2:15 p.m.	No insulin	No insulin	4 units insulin	5 units insulin	5 units insulin
2:25 p.m.	Coma	Coma	Coma	Coma	Coma
2:30 p.m.	All breathe noisily, very sick				
3:45 p.m.	Lifts head	Sits up	Coma	Coma	Coma
4:00 p.m.	Lifts head	Active	Coma	Coma	Coma
4:55 p.m.	Sits up	Active	Paralyzed	Head up	Coma
Next morning	Animals all healthy				

Since the toxic effects of alcohol and acetone are roughly proportional to its concentration in the blood, it is evident that insulin does not accelerate the oxidation of alcohol or of acetone. These results are in harmony with the experiments of T. Brailsford Robinson and A. B. Anderson, who

found that insulin does not affect the oxidation of diacetic acid injected into animals.[1]

It is therefore probable that insulin exerts its effects in the first steps rather than upon the last steps in the oxidation of fats and sugars in the body.

SUMMARY AND CONCLUSIONS

From these experiments it would appear that insulin does not increase the oxidation of alcohol or acetone in the body or antagonize their toxic effects.

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[1] We have not been able to secure a copy of Doctor Robertson's original article.

EXPERIMENTAL TETANY AND DIET

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It has been known for many years that after thyroparathyroidectomy dogs show in a few days certain symptoms of a violent motor type generally known as tetany, and the great majority of the animals die very soon thereafter. Attempts to alleviate these symptoms by diet or special therapeutic measures have been unsuccessful until very recent years, except that the administration of certain salt solutions, especially calcium salts, may cure the symptoms temporarily. The animals that occasionally survive the operation are usually considered to have accessory parathyroid tissue in some other region of the body.

In 1922 Dragstedt and his co-workers (1) reported successful attempts to prevent the onset of tetany in thyroparathyroidectomized dogs by adding large amounts—from 50 to 100 grams—of lactose daily to the diet. They were convinced, in view of many facts concerning the toxicity of the products of intestinal putrefaction, that the efficacy of this preventive measure was due to the resultant acidic change of intestinal contents which checked the development of proteolytic bacteria and accordingly suppressed the formation of toxic substances which they thought to be responsible for the tetany.[2] It has been our plan to repeat Dragstedt's work under more careful dietary control, taking advantage of Cowgill's method of feeding (2), in which the diet consists of more or less purified food substances, such as casein for protein, sucrose for carbohydrate, lard for fat, butter fat for vitamin A, yeast extract for vitamin B, bone ash for roughage, and a suitable salt mixture for mineral constituents.[3] By

[1] International Fellow of the Rockefeller Foundation.

[2] For a discussion of the pathogenesis of tetany see W. G. MacCallum, Medicine, 1924, iii, 137. The calcium factor is discussed by H. A. Salvesen, Acta Scand. Med., 1923, Suppl. C.

[3] Karr's salt mixture:

Sodium chloride	10 grams
Calcium lactate	4 grams
Magnesium citrate	4 grams
Ferric citrate	1 gram
Lugol's solution	a few drops

The amounts of bone ash and salt mixture in 100 grams of food (about 480 calories) were 2.3 and 1.2 grams, respectively.

doing so we have been able to vary the foodstuff components at will both quantitatively and qualitatively, keeping the total caloric value nearly the same, and to observe the effects of various diets in affecting the onset of experimental tetany in dogs. During the experiments, the blood

TABLE 1

Relation of diet to the appearance or non-appearance of tetany after thyroparathyroidectomy

TETANY		"BOUNDARY"		NO TETANY	
Number of dogs	Diet	Number of dogs	Diet	Number of dogs	Diet
5	Standard: Casein.....37.6% Sucrose.....34.9	3	Lactose *d:* Casein..... 5.5% Lactose..... 5.0 Sucrose.....62.1	3	Lactose *a**: Casein..... 5.5% Lactose.....68.1
4	High glucose: Casein..... 5.5 Glucose.....68.1	2	Casein-lactose *d:* Casein.....20.0 Lacotse..... 5.0 Sucrose.....53.1	2	Lactose *b:* Casein..... 5.5 Lactose.....26.6
1	Sucrose-glucose: Casein..... 5.5 Glucose.....27.0 Sucrose.....41.1	3	Casein-lactose *b:* Casein.....40.0 Lactose.....15.0 Sucrose.....23.1	4	Lactose *c:* Casein..... 5.5 Lactose.....15.0 Sucrose.....53.1
4	High sucrose: Casein..... 5.5 Sucrose.....68.1			4	Casein-lactose *a:* Casein.....37.6 Lactose.....34.9
3	High dextrin: Casein..... 5.5 Dextrin.....68.1				
3	Casein-lactose *c:* Casein.....40.0 Lactose..... 5.0 Sucrose.....33.1				

* Merck's lactose: Calcium content of 100 grams of this product is only 7 to 8 mgm. as CaO.

sera of the animals were analyzed for calcium and phosphorus usually once or twice a week, and in some cases several days in succession. Tisdall's method was used for calcium, and Briggs' modification of the Bell-Doisy procedure for phosphorus.

EXPERIMENTAL. After the animals were fed on the selected diet for 6 to 7 days, they were operated upon and the same diet was continued. Severe tetany, when it occurred, usually developed on the second or the third day following the operation. After the animals developed tetany,

TABLE 2

Effects of parenteral administration of calcium lactate and oral administration of lactose in the treatment of tetany

DOG	EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	TREATMENT	REMARKS
		kgm.	*gm.*		
I	25	10.8	120	Standard diet	Operation
	27	10.8	18	2 p.m. 30 gm. lactose	1:30 p.m. severe tetany
	28	10.4	15	50 gm. lactose	Tetany
	29	10.1	0	10:30 a.m. 30 gm. lactose	
				5:30 p. m. 4 gm. Ca-lactate (1%) subcutaneously	3:30 p.m. tetany
	30	10.0	0	10:30 a.m. 4 gm. Ca-lactate intravenously	
				5 p.m. 4 gm. Ca-lactate intravenously	9:30 p.m. tetany
	31	9.7	0	10 a.m. 4 gm. Ca-lactate intravenously	3 p.m. died
II	6	7.7	110	Standard diet	Operation
	9	7.6	110	5 p.m. 30 gm. lactose high lactose diet	4 p.m. severe tetany
	10	7.4	35	30 gm. lactose	Deep depression
	11	7.5	53	30 gm. lactose	Depression
	12	7.4	70	10:30 a.m. 20 gm. lactose	
				3 p.m. 3 gm. Ca-lactate intravenously	2:20 p.m. tetany
	13	7.3	6	30 gm. lactose	Depression No motor symptoms
	14	7.3	0	20 gm. lactose	
				3 gm. Ca-lactate subcutaneously	9 a.m. tetany
	15	7.2	0	30 gm. lactose	Depression
	16	7.1	0	35 gm. lactose	Depression
	20	6.9	0	35 gm. lactose	5 p.m. died

attempts were made in various ways to cure them; and some of those which were relieved by the treatment again became the subjects for later experiments. In the treatment, special attention was paid to the efficacy of lactose and calcium lactate. If an animal did not show tetany under the

TABLE 3

Effects of new method with oral administration of calcium lactate and lactose in the treatment of tetany

DOG	EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		TREATMENT	REMARKS
				Ca	P		
		kgm.	*gm.*	*mgm.*	*mgm.*		
VIII	24	6.5	0	4.5	7.8	1 p.m. 100 cc. subcutaneously {0.3% Ca-lactate, 0.6% NaCl}	11:20 a.m. tetany
						2:30 p.m. 400 cc. milk, 50 gm. lactose	
						High lactose diet	
	25	6.5	85			High lactose diet alone	No tetany
	26	6.4	85			High lactose diet alone	No tetany
	27	6.4	85			High lactose diet alone	Recovered
	97	6.5	Ad lib.				Dog biscuit given
	98	6.5	0	5.5	6.6	4 p.m. 10 gm. Ca-lactate by sound	3 p.m. tetany
						Lactose *b* diet	
	100	6.3	85			Lactose *b* diet	Recovered
IX	43	9.3	0				Beginning tetany
	44	9.1	0	5.5	7.1	140 cc. {0.3% Ca-lactate, 0.6% NaCl} subcutaneously	Severe tetany
						400 cc. milk; 15 gm. lactose	
	45	9.0	0			400 cc. milk; 40 gm. lactose	No tetany
	46	8.9	115			Lactose *b* diet alone	
	48	8.8	115			Lactose *b* diet alone	Recovered
XI	18	6.1	50	4.7	10.5	6 p.m. 140 cc. subcutaneously {0.3% Ca-lactate, 0.6% NaCl}	5 p.m. severe tetany
						10 gm. Ca-lactate, 15 gm. lactose } by sound	
						Lactose *b* diet	
	19	6.0	80			Lactose *b* diet alone	Recovered

TABLE 4

Two interesting cases in the treatment of tetany

DOG	EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		TREATMENT	REMARKS
				Ca	P		
		kgm.	*gm.*	*mgm.*	*mgm.*		
XII	18	5.3	0	5.7	7.8	11:30 a.m. 10 gm. Ca-actate by sound	a.m. severe tetany
	19	5.2	0	6.1	8.2	10 gm. Ca-lactate by sound	Depression
	20	5.2	0	5.8	10.6	10 gm. Ca-lactate by sound	Tetany
	21	5.0	0			10 gm. Ca-lactate + 15 gm. lactose by sound	Tetany
	22	4.9	72	9.1	5.9	10 gm. Ca-lactate + 15 gm. lactose by sound	No tetany
	23	4.9	70	8.5	11.3	15 gm. lactose	No tetany
	24	4.8	30	6.2	6.4	15 gm. lactose	Depression
	25	4.7	3	5.6	9.1	15 gm. lactose	Tetany
	26	4.6	0	5.1	8.7	15 gm. lactose	Tetany
XVII	26	6.1	20			10:30 a.m. 12 gm. Ca-lactate by sound	a.m. severe tetany
	27	6.0	10			10 gm. Ca-lactate + 15 gm. lactose by sound	Depression
	28	5.8	3			10 gm. Ca-lactate + 15 gm. lactose by sound	Tetany
	29	5.8	0			10 gm. Ca-lactate + 15 gm. lactose by sound	Tetany
	30	5.6	0			200 cc. Ringer's solution twice a day, subcutaneously; 10 gm. Ca-lactate + 15 gm. lactose by sound	
	31	5.5	20			10 gm. Ca-lactate + 15 gm. lactose by sound	No tetany
	33	5.5	58			15 gm. lactose	Recovered

Protocol 1

Dog No. VIII, ♀, 7.1 kgm.

EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		DIET AND TREATMENT	REMARKS
			Ca	P		
	kgm.	*gm.*	*mgm.*	*mgm.*		
1	7.1	85			High glucose	
			11.2	3.8		
7	7.2	85			High lactose	Operation
22	6.7	85			High glucose	Spasticity in hind legs
23	6.6	0	4.5	7.8	Ca-lactate + lactose	Severe tetany
24	6.5	85			High lactose	Slight jerky movement
						No tetany
32	6.4	85			Lactose *b*	
			10.1	5.1		No tetany
55	5.9	90			Lactose *c*	
			9.8	4.5		No tetany
62	6.0	93			Lactose *d*	
						No tetany
67	6.1	98			Casein-lactose *d*	
						No tetany
72	6.3	95			Casein-lactose *c*	
						No tetany
79	6.5	95			Standard	
						No tetany
83	6.5	95			Meat diet	
						No tetany
			10.2	6.1		
88	6.6	Ad lib.			Dog biscuits	
97	6.5					Muscular spasmodic contraction
98	6.3		5.5	6.6	Ca-lactate + lactose	Tetany
99	6.3				Lactose *b*	
						No tetany
105	6.3	85	10.2	6.5	Standard	
114	5.8	30				Losing appetite
115	5.7	10				
116	5.7	11				
117	5.6	0			Lactose	Tetany
118	5.5	53			Casein-lactose *a*	
137	5.5	85				Very weak; losing weight
149	5.1	10				Failure of appetite; emaciation; sunken eyes; low temperature; died
			11.3	5.9		
156	4.6	0				

experimental conditions first selected, the diet was changed until symptoms of tetany appeared; this was done in order to exclude any possible action of accessory glands in the experiments.

In table 1 are given the results obtained with various diets. In each case all constituents of the food mixture except the casein and carbohydrate were kept constant.

Protocol 2

Dog No. IX, ♂, 9.5 kgm.

EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		DIET AND TREATMENT	REMARKS
			Ca	P		
	kgm.	*gm.*	*mgm.*	*mgm.*		
1	9.5	115			Lactose *b*	
			10.5	4.3		
8	9.5	115				Operation
			8.1	6.4		No tetany
31	9.5	115			Lactose *c*	
			7.8	7.5		No tetany
38	9.4	115			Lactose *d*	
42	9.4	100				Muscle twitching
43	9.3	0	5.5	7.1	Ca-lactate + lactose	Tetany
44	9.1	85			Lactose *c*	
						No tetany
48	8.9	115			Lactose	Beginning tetany
49	8.8	115			Casein-lactose *a*	
						No tetany
55	8.7	2	5.8	6.5	Ca-lactate	Tetany
59	8.4	0			Ca-lactate	Tetany
61	8.1	0			Ca-lactate	Tetany
64	7.6	0			Ca-lactate + lactose	Tetany
72	7.4	0			Ca-lactate + lactose	Tetany
82	7.3	110			Ca-lactate	Mild tetany
						No tetany
111	7.5	70				
112	7.4	0				No tetany; stops eating
113	7.2					Found dead

The food was given to the dogs at 4 p.m., the blood samples for analyse usually being withdrawn before the meal.

As will be seen in table 1, with the diets: standard, high glucose, sucrose-glucose, high sucrose, and high dextrin, the animals developed severe

symptoms of tetany on the second or the third day following the operation, while conversely with the lactose diets, *a*, *b* and *c*, no manifest tetany occurred. When the food was changed from lactose to another diet for the operated dogs which had been kept free from tetany by lactose feeding, tetany usually appeared within 24 hours. When the amount of lactose in

Protocol 3

Dog. No. XI, ♀, 6.4 kgm.

EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		DIET AND TREATMENT	REMARKS
			Ca	P		
	kgm.	*gm.*	*mgm.*	*mgm.*		
1	6.4	80			Lactose *c*	
			10.3	3.0		
7	6.3	80				Operation
			7.5	5.6		No tetany
12	6.2	80			Lactose *d*	
						No tetany
17	6.2	80			High sucrose	
18	6.1	50	4.7	10.5	Ca-lactate + lactose	Severe tetany
19	6.0	80			Lactose *b*	
			7.3	9.9		No tetany
26	5.7	85			Lactose *d*	
						No tetany
32	5.8	85			Casein-lactose *c*	
						No tetany
35	5.5	0	4.7	9.4	Ca-lactate + lactose	Tetany
36	5.5	85			Casein-lactose *a*	
						No tetany
41	5.7	85			Casein-lactose *b*	
			7.5	5.8		No tetany
48	5.8	85			Casein-lactose *c*	
						No tetany
60	6.2	85			Standard	
						No tetany
69	6.5	0			Lactose	Tetany
70	6.4	85			Casein-lactose *a*	
						No tetany
90	6.6	85			Standard	
						No tetany
113	6.6	85				

the foods was cut down to 5 per cent (lactose *d*), the animals started to show the signs of tetany. On those diets listed under the column "boundary," most animals developed tetany after a slight delay 3 to 5 days following the dietary change; and their symptoms were also usually chronic. Some dogs that were less susceptible to tetany in certain conditions (which will be discussed later) did not show any adverse symptoms. It is also

evident in the table that when the amount of casein in foods was relatively low (lactose *c*, 5.5 per cent casein), the amount of lactose which could prevent the onset of tetany was comparatively small (10 to 15 per cent). This fact may serve to give one explanation for the long discussed question why milk acts beneficially in preveting tetany. When the casein was increased more lactose was needed (casein-lactose *a*, *b*, c).

The dextrin experiments were significant, because lactose and dextrin are commonly supposed to act in somewhat the same way as far as the acidophilic change of the intestinal flora is concerned.

Protocol 4

Dog No. XV, ♀, 4.6 kgm.

EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		DIET AND TREATMENT	REMARKS
			Ca	P		
	kgm.	*gm.*	*mgm.*	*mgm.*		
1	4.6	55			High dextrin	
			11.5	3.7		
7	4.5	55				Operation
9	4.4	55				Beginning tetany
10	4.4	55	6.1	6.8	Ca-lactate + lactose	Severe tetany
11	4.3	55			Lactose *b* + Ca-lactate	
						No tetany
16	4.3	0			Ca-lactate	Tetany
						No tetany
21	4.2	50			Ca-lactate	Tetany
						No tetany
26	4.3	55			Lactose	Mild tetany
39	4.4	55			Casein-lactose *a*	
						No tetany
48	4.5	55			Standard	
49	4.5	55			Ca-lactate + lactose	Tetany
50	4.5	65			Casein-lactose *a*	
						No tetany
65	4.5					

Three tables (2, 3, 4) and several typical protocols (1, 2, 3, 4) of the experiments are presented here as a preliminary to the discussion of the following points:

1. Methods of treatment of manifest tetany.

2. Content of calcium and phosphorus in the blood sera of thyroparathyroidectomized dogs.

3. Readjustment and change in susceptibility of thyroparathyroidectomized dogs according to the length of time after the operation.

EFFECTS OF ADMINISTRATION OF CALCIUM LACTATE AND LACTOSE IN THE TREATMENT OF DOGS WITH TETANY. In dogs with tetany the administration of calcium lactate and lactose was carried out in various ways, parenterally and orally, with the purpose of finding out the most effective method of treatment. The results obtained can be summarized as follows:

1. Parenteral administration (subcutaneous and intravenous injection) of calcium salts was immediately effective in stopping the severe symptoms of tetany. However, most of the dogs treated this way suffered from the failure of appetite and depression, and eventually died in a few weeks (table 2). Another familiar great disadvantage of this method was that the injection must be repeated once or twice every day.

2. Although lactose administration by mouth alone, at the time severe symptoms developed, appeared to give some relief to the animals after a few hours, it was never so successful as the administration of calcium salts in curing symptoms (table 2).

3. However, by stopping the symptoms of tetany by oral administration of calcium lactate (1.5 to 2 grams per kilo body weight) and then changing the diet to a food mixture rich in lactose, we have been able usually, without giving any more calcium, to save the animals from the recurrence of tetany. In case the oral administration was impossible because the animals vomited, we injected subcutaneously 100 to 300 cc. of an isotonic solution which consists of 0.3 per cent calcium lactate and 0.6 per cent sodium chloride. This injection always stopped the severe tetanic symptoms, and the oral administration of calcium lactate and the dietary change to lactose foods were successful after a few hours (table 3).

4. In a few cases, when the result of calcium lactate administration alone was not satisfactory, we noticed that the combined use of calcium lactate and lactose was more effective, one blood analysis indicating that the combined use increased the calcium level in the blood serum, whereas the calcium administration alone failed to do it (table 4, case XII).

5. In two cases among more than twenty, even the combined administration of calcium lactate and lactose failed to save the animals from the failure of appetite and depression accompanied with chronic motor symptoms. The calcium of the blood serum in these cases was never brought up to normal. It was also worth while to note that one of these cases was finally saved by using, first, Luckhardt's method (3), (injection of 200 cc. of Ringer's solution twice a day) and then following with the combined use of calcium and lactose (table 4, case XVII).

6. It appears that dogs exhibiting tetany of the severe motor type, but with less depression and good appetite are much more easily cured by the administration of calcium salts and lactose, than those with less severe motor symptoms, but with deep depression.

CALCIUM AND PHOSPHORUS IN THE BLOOD SERUM. On the whole, our results (tables 3, 4; protocols 1 to 4) were in accord with thoseobtained by the earlier investigators: Salvesen (4), Hastings and Murray (5):

> In every case whenever the animal showed the symptoms of tetany after thyroparathyroidectomy, the calcium content of serum was less than 7 mgm. in 100 cc., compared with 10 to 11 mgm. in normal sera; and a parallelism existed between the degree of lowering of the calcium and the violence of the symptoms. In severe cases, the amount of calcium was usually as low as 4 to 5 mgm. in 100 cc. of serum. When the animals were kept from manifest tetany by a lactose diet, the calcium content of blood serum at first showed a value slightly lower than normal, usually around 8 to 9 but never below 7 mgm.; and this slightly lower value showed a tendency to rise gradually to normal in the course of time after the operation. On the other hand the phosphorus content of blood serum in thyroparathyroidectomized dogs was always increased, even if the animals were kept free from tetany by lactose feeding or by calcium administration. This increased phosphorus returned gradually to normal in the course of time.

SUSCEPTIBILITY OF THE THYROPARATHYROIDECTOMIZED DOGS TO TETANY AND THE IDEA OF DISPENSABILITY OF PARATHYROID GLANDS. Two facts in this connection which were already pointed out by Dragstedt and Luckhardt (1), (6) have been fully confirmed in our experiments:

1. It is much easier to prevent the appearance of tetany or depression in tetany than it is to cure it, once it has become established.

2. When the thyroparathyroidectomized dogs are kept free from tetanic symptoms by suitable treatment, they become gradually less susceptible to this condition in the course of time. For instance as will be seen in protocols 1 to 4, in cases VIII, IX and XV, the animals were able to remain in good condition on diets with less lactose according to the length of time after the operation, so that in 50 to 70 days after the operation they could live on the normal standard diet, containing no lactose, without showing any adverse symptoms. However, when once tetany has become established, the animals become very susceptible again. This was clearly shown in cases IX, XI and XV. In cases IX and XV the animals needed calcium administration several times in a few weeks after the first onset of tetany (case IX, exptl. day 48 to 82; and case XV, exptl. day 11 to 26). This indicates that some readjustment may be at work in the body after the loss of the thyroids and parathyroids to compensate their functions.

This was shown in another way by the fact that calcium and phosphorus in blood serum returned gradually to normal in the course of time. Nevertheless this readjustment can never be construed as evidence for the support of the idea of dispensability of parathyroid glands. For, even the animals which were kept free from tetany more than 50 to 70 days (4 cases) and which appeared to be normal, developed tetany under certain conditions, known and unknown; and when once they developed

tetany, this readjustment seemed to have been destroyed with the consequence that the animals became as susceptible as they had been soon after the operation. While we could not see any particular reason of the recurrence of tetany in cases VIII and XI (case VIII, exptl. day 98; case XI, exptl. day 69), it should not be overlooked that the body weight of both animals showed a noticeable increase in a short time, which was apparently not due to edema.

THE MECHANISM OF THE PREVENTION AND CURE OF THE SYMPTOMS OF TETANY BY FEEDING LACTOSE. The experimental data described above show apparently that lactose administration by mouth not only prevents the onset of tetany, but is beneficial in any stage of the disease; and the

TABLE 5

TETANY		NO TETANY	
Number of dogs	Diet	Number of dogs	Diet
2	High sucrose diet + 6 gm. lactose (20% solution) per kilo body weight subcutaneously	2	Galactose diet: Casein..... 5.5% Galactose.....20.0 Sucrose.....48.1
2	High sucrose diet + 4 gm. galactose (20% solution) per kilo body weight subcutaneously	3	Lactose diet *a* and *c*; "cellu flour" in place of bone ash
3	High sucrose diet or Standard diet + castor oil (30 to 50 cc.) given 1 to 2 hours after meal		

manner of its action appears to be a "specific" one. In order to get further light on this point a few experiments were carried out as follows:

1. Parenteral administration of lactose and galactose.
2. Evacuation of bowel by a laxative agent (castor oil).
3. Galactose administration by mouth.
4. Lactose diets, in which the bone ash containing calcium salts was replaced by "cellu flour" as "roughage."

The results are summarized in table 5.

As will be seen in the table, tetany was not averted by the parenteral administration of both lactose and galactose nor by laxation with castor oil, while a positive result was obtained with the administration of galactose by mouth. The "cellu flour" experiment exactly paralleled that of the customary lactose diet, containing bone ash, thus showing that the calcium of the bone ash had nothing to do with the prevention of the onset of tetany.

TABLE 6

Influence of lactose diets on the calcium and phosphorus of the blood serum in normal dogs

DOG	EXPERIMENTAL DAY	WEIGHT OF DOG	WEIGHT OF FOOD EATEN	IN 100 CC. SERUM		DIET
				Ca	P	
		kgm.	*gm.*	*mgm.*	*mgm.*	
XIV	1	4.1	60			Standard diet
	6	4.2	60	10.5	5.0	
	7	4.2	60	11.0	4.2	
	8	4.2	60			Casein-lactose *a* diet
	11	4.2	60	13.2	5.8	
	12	4.2	60	12.7	5.5	
	13	4.2	60			Standard diet
	16	4.1	60	10.5	4.1	
	17	4.2	60	11.2	4.5	
	18	4.2	60			Lactose *b*
	22	4.2	60	13.8	4.5	
	23	4.2	60	13.5	5.9	
	24	4.2	60			High dextrin diet
	26	4.1	60	10.2	4.3	
	27	4.1	60	9.5	3.7	
XVII	1	6.9	90			Standard diet
	5	6.9	90	12.4	5.1	
	6	6.9	90	12.2	7.5	
	7	6.8	90			Lactose *b*
	11	6.7	90	12.5	5.3	
	13	6.7	90	13.1	5.7	
XVIII	1	6.5	85			Standard diet
	5	6.5	85	12.0	6.1	
	6	6.5	85	11.6	5.5	
	7	6.5	85			Lactose *b*
	10	6.3	90	12.7	5.4	
	11	6.4	90	13.4	5.3	

THE INFLUENCE OF LACTOSE DIETS ON THE CONTENT OF CALCIUM AND PHOSPHORUS IN THE BLOOD SERUM OF NORMAL DOGS. The results obtained are presented in table 6.

The lactose diet, when fed to normal dogs, seems to increase the calcium content of blood serum slightly. In the case of the phosphorus value no noticeable change was observed.

DISCUSSION

From these experiments it appears that the presence of certain amounts of lactose and galactose in the food served to prevent the onset of tetany in the dogs indefinitely, whereas when the animals were fed with other carbohydrates, they devloped severe tetany in a few days. The amount of lactose needed for this preventive effect was relatively smaller than has been generally believed necessary to secure an acidophilic change of the intestinal flora. It was shown also that when the protein (casein) in the foods was increased, more lactose was needed to permit a beneficial effect. The fact that parenteral administration of lactose and galactose failed to prevent tetany seems to suggest that the place where lactose acts may be sought in the alimentary tract. This is again supported by the fact that calcium lactate given by stomach sound apparently was more effective when it was used together with lactose. However we should not be too hasty in attributing the effect of lactose to the acidic change of the intestinal contents, until the study of the pH of the intestinal contents has been made; for it was also found that the high dextrin diet was not effective in preventing tetany, although it has been found to favor acidophilic conditions. On the other hand the galactose prevented tetany. Various possibilities as to the way in which the gastro-intestinal tract may function in the prevention of tetany present themselves; and much more investigation is needed on this point.[4]

The writer's sincere thanks are due to Prof. Lafayette B. Mendel for his kind advice and criticism throughout this work.

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THE EFFECT OF OVARIAN EXTRACTS UPON THE SPONTANEOUS CONTRACTIONS OF THE FALLOPIAN TUBE OF THE DOMESTIC PIG WITH REFERENCE TO THE OESTROUS CYCLE

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Since the time of the publication of Brown-Sequard's (1) studies upon the internal secretion of the testicles, it has been more or less generally conceded that the ovary is an organ of internal secretion as well as of reproduction, and that its rôle as such is directly concerned in maintaining the integrity of the other generative organs. Great impetus was given to this belief when Fraenkel (2) in 1910 showed that in women the next succeeding menstrual period failed to occur when the corpus luteum had been destroyed by means of a cautery. In recent years a great deal has been published upon the subject but at the present time little is known of the chemistry and physiological activity of the ovary.

A number of workers have tried the action of ovarian extracts upon the musculature of the reproductive tract, but with few exceptions quantitative methods have not been used. Moreover, all of these tests antedated the knowledge of the cyclic variations which underlie the spontaneous contractions of the uterus and Fallopian tube.

For some time the writer has been engaged in the study of the tubal contractions of the domestic pig, the Macacus Rhesus monkey and the human, with special reference to the cyclic variation in the types of contraction which occur in species so widely separated. Briefly stated, these studies show that two definite types of spontaneous contraction occur during the ovulation cycle. One type occurs at the time of ovulation and is characterized by rapid contraction (8 to 15 per minute), with a marked tendency toward periodic alternation in amplitude. In the other type the contractions are slower (about 4 per minute) and of uniform amplitude.

The present study embraces a consideration of the action of ovarian extracts upon the contractions of the Fallopian tube of the domestic pig with reference to the oestrous cycle. The tube of the pig was selected for these studies for the following reasons: First, through the contribution of Corner (3) it is readily possible to follow in material obtained directly from

the abattoir the anatomical changes in the uterus and ovary which underlie the reproductive cycle; second, cyclic variations in tubal contractions were first demonstrated on the basis of Corner's contribution; third, it is known that the cyclic variations in tubal contractions follow similar patterns in species so widely separated as the domestic pig and Macacus Rhesus; fourth, tubal contractions vary little in character, being either typical of oestrus or interoestrus, except in border line cases where one type is undergoing conversion into the other, while the uterus, as illustrated in the work of Keye (4), is subject to a greater number of individual contraction patterns during an entire oestrous cycle.

METHOD. The extracts employed in these experiments consisted of *a*, stock solutions of commercial preparations of corpus luteum, and *b*, of a series of extracts prepared in this laboratory from the different components of the ovary, namely, the corpora lutea, the large follicles, the recently ruptured follicles, and the stroma or body of the ovary of swine. The method of preparation briefly stated is as follows:

Iced glands coming directly from the packer were examined macroscopically and separated into their component parts. Those glands which contained large flesh-colored corpora lutea were reserved for one extract of the series; those consisting of follicles measuring from $7\frac{1}{2}$ to 10 mm. for another, and those of the blood containing recently ruptured follicles for another. The corpora lutea, the large follicles and the recently ruptured follicles were carefully dissected from the ovaries and placed in individual containers, the remaining part or ovarian residue being reserved for another fraction. These portions were at once comminuted into a fine pulp, spread out in thin layers over the surface of thin drying pans and placed in a drying apparatus at a temperature of 38°C. In less than six hours the masses were completely dry. The different portions were then ground to a very fine powder, the final procedure before extraction.

As an initial procedure cold physiological saline extracts were prepared, representative of each of the four parts of the pulverized gland. Other portions were extracted successively with petroleum ether, acetone, alcohol and ether in the Soxhlet apparatus. After extraction with each solvent, a portion of the material was removed from the Soxhlet tube, before the next solvent was applied. These removed portions were subjected to reëxtraction with physiological saline solution, after which each extractive was adjusted to correspond to 10 per cent of the dried gland substance by weight, the final procedure before testing. All extracts of the series used in the test represent the physiological salt solution product of the gland. The purpose in using lipoidal solvents was to remove, if possible, probable active substances from the gland portions before final extraction with physiological saline solution.

At a later stage portions of all physiological saline extracts were deproteinized in accordance with the lead acetate method. Excess of lead was subsequently removed with sodium bicarbonate, which leaves only sodium acetate and sodium chloride in solution. Because of possible interference on the part of the sodium acetate upon the spontaneous tubal contractions, the amount of sodium acetate in solution was quantitatively determined, and a sodium acetate solution in normal saline solution of the same strength and adjusted to the pH of the deproteinized corpus luteum extract, was made to serve as a control.

Saline extracts of fresh corpus luteum substance were also prepared by macerating the same with cold physiological salt solution, allowing the suspension to stand for several hours before filtering.

In order to determine the effect of heat upon corpus luteum extracts, an active extract was boiled for two hours.

As a control experiment a physiological saline extract of powdered, dried and defatted beef was prepared in accordance with the method described for the preparation of the ovarian extracts.

Because of the possible influence of calcium and potassium upon the tubal contractions the actual amount of both substances in the active corpus luteum extract was determined. A definite amount of the extract was also charred to ash, after which the residue was dissolved in HCl and brought back to neutrality, the purpose being to determine whether the inorganic salts in the original corpus luteum extract have any influence upon tubal contractions.

The apparatus used in these experiments was the ordinary kymographion, set to revolve once in sixty minutes, to which a time clock recording one-half minute intervals was attached. Rings of Fallopian tube muscle, about 3 mm. in length, were suspended in 50 cc. of oxygenated Locke's solution and the former attached by means of a string to a recording lever. Care was taken to maintain a constant temperature of 37.5°C. throughout the experiments.

As an initial procedure normal spontaneous contractions were first obtained in all instances before addition of ovarian extracts. As suggested in a former paper, considerable time elapses, frequently about one-half hour, before the contractions become active and regular. When such stages were reached ovarian preparations were added to the normally contracting tube. As a matter of routine, 0.5 cc. of a 1:10 solution was added first. If this met with no muscular response within 5 to 10 minutes, progressively increasing doses were added at definite intervals of 5 to 10 minutes.

Finally, because of the possibility of obtaining bizarre types of contraction, the point must be emphasized that the preparation of a satisfactory

Locke solution is a most important factor in the successful control of spontaneous contractions of tubal musculature. It has been found that some chemicals are entirely unsuited and throughout these experiments Locke solution which failed to produce the characteristic contractions of oestrus or interoestrus in control experiments was discarded. Other factors that may cause bizarre types of contractions are *1*, old or bacterially contaminated solutions; *2*, impurities in distilled water and in the inorganic salts. Triple distilled water or ordinary laboratory distilled water redistilled with $KMnO_4$, to which a stick of sodium hydroxide has been added, is preferable to ordinary distilled water.

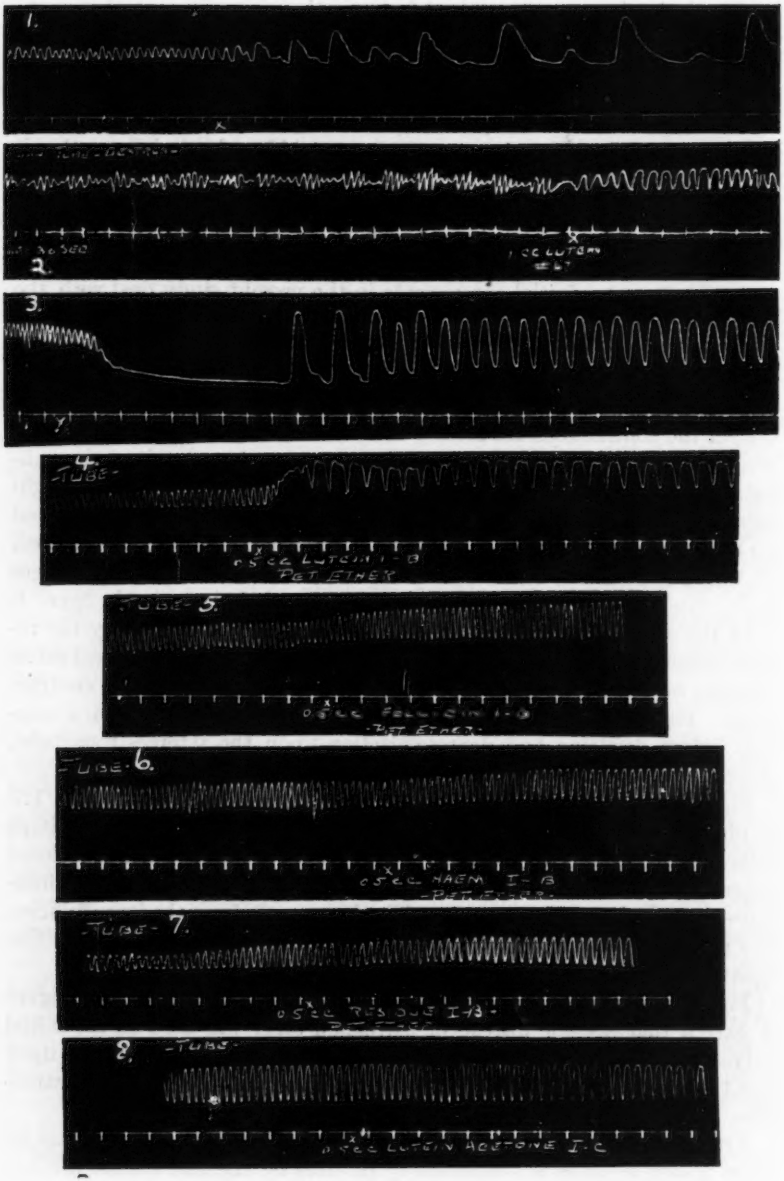
Observations. Initial experiments in the present study deal with the effects of commercial extracts of corpus luteum upon the Fallopian tube of the pig during the oestrous and interoestrous periods, as represented in figures 1 and 2. Figure 1 shows the effect of 1 cc. of a 1:5 solution of a commercial extract upon the interoestrous tube. After addition of the extract the contractions are greatly increased in amplitude, with a correspondingly slower rate, but the same general pattern is maintained throughout. Figure 2 represents typical tubal contractions of an animal slaughtered while in heat. The characteristic oestrous pattern is maintained up to the point where 1 cc. of the same extract referred to above is added. At this stage, in contradistinction to the effects above, the pattern changes to the type characteristic of the interoestrous period illustrated in figure 1.

Of the series of extracts prepared and tested in this laboratory the results obtained are most interesting. In the first place, physiological saline extracts of dried and pulverized corpus luteum influence tubal contractions. Figure 3 shows the effect of 1 cc. of a 1:5 dilution of such a solution before defatting and deproteinization upon the interoestrous tube, where the results are analogous to the results obtained in figure 1.

Figures 4, 5, 6 and 7 represent respectively the effects of 1 cc. of a 1:5 of physiological saline extracts from the pulverized corpora lutea, the large follicles, and the stroma, prepared after an inital extraction with petroleum ether. These records show that the corpus luteum extract actively stimulates the tube in 0.5 cc. amounts, while the extracts from the large follicles, the ruptured follicles, and the stroma produce little if any effect in like amounts.

Figure 8 represents the same corpus luteum preparation tested in figure 4, except that acetone instead of petroleum ether was used as the initial solvent. In 0.5 cc. amounts the saline extracts show little influence upon the tubal contractions; apparently the result is due to the initial extraction with acetone.

The effect of a deproteinized physiological saline extract of corpus luteum is seen in figure 9. Removal of proteins by the lead acetate method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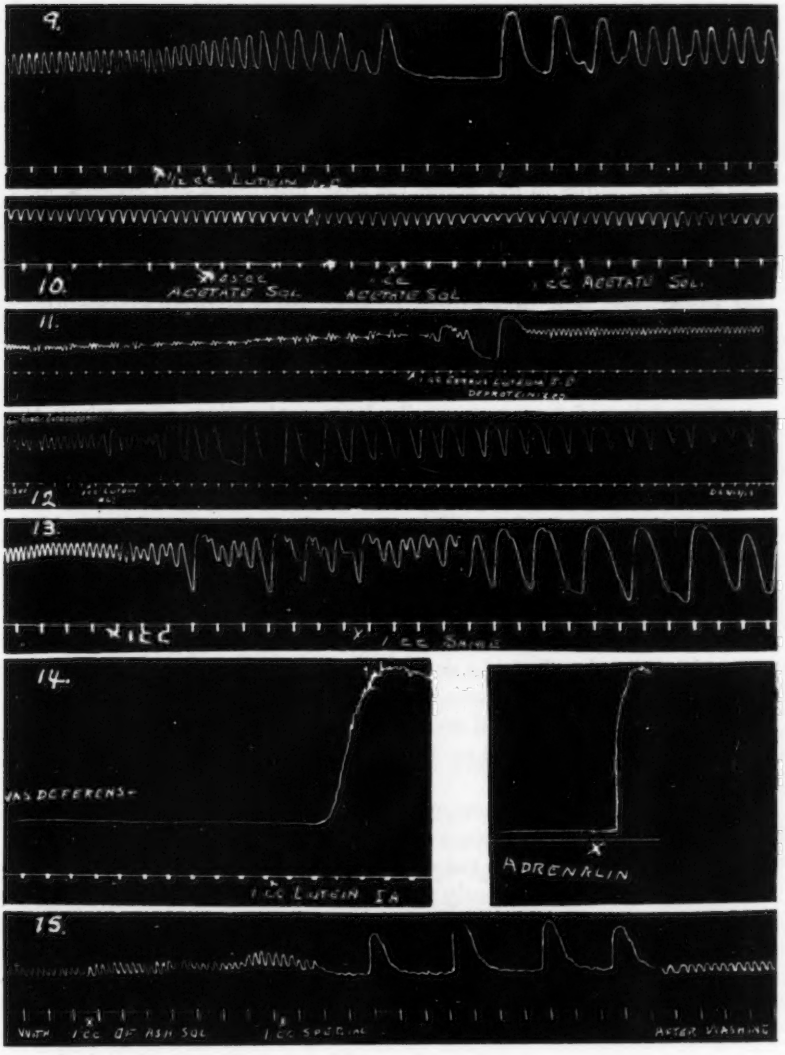
PET ETHER
TUBE 7.
0.5 CC RESIDUE I-B
TUBE
0.5 CC LUTEIN HORMONE I-C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does not lessen the activity of the extract. On the other hand, it appears that by freeing the solution of proteins, the extract actually increases in potency, as illustrated in the graph where 0.5 cc. of the extract shows a powerful stimulating effect upon the tube. That the sodium acetate in solution is not responsible for this stimulation is shown in figure 10, where a normal saline solution containing the same amount of sodium acetate by weight and adjusted to the same pH in doses five times as great, has no effect upon the tube.

Of all the records in our series the most interesting and striking were those in which there is a change of the contraction pattern after addition of deproteinized physiological saline extracts of corpus luteum. The first half of figure 11 illustrates the typical undulatory contractions that occur only at oestrus. The tube in this instance came from an animal whose ovaries showed very recently ruptured folllicles. The record shows that upon addition of 1 cc. of the deproteinized extract after a short period the spontaneous contractions cease altogether. There is a period of marked relaxation followed by an unusually large contraction, after which the pattern is sharply changed to the interoestrous type and remains such throughout. While it was not possible to obtain a complete change of the contraction type in every instance, still such changes were observed in a number of cases. In no instance was the writer ever able to obtain such a change when the interoestrous tube was used. Extracts from the mature follicles, recently ruptured follicles and the stroma of the ovary have always given negative results even when larger amounts are used.

The active substance in the corpus luteum extracts is thermostable. Figure 12 shows the effect of a deproteinized extract upon the interoestrous tube. The active solution was boiled for two hours, and that it has retained all or a greater part of its activity is seen in figure 13. It required, however, a double dose or 2 cc. to produce effects comparable to those in figure 12.

Figure 14 is shown in connection with the work of Matsumoto and Macht who found in the corpus luteum some substance which they maintain has a selective action upon the vas deferens of the rat. Deproteinized solutions of the follicular, recently ruptured follicular and stroma extracts have some slight stimulating effect upon the vas, but small in comparison with that of an analogous corpus luteum extract. The latter, judged from the height of contraction, is as potent upon the vas as epinephrin in 1:100,000 dilution.

Figure 16 shows the effect of a potent deproteinized solution of corpus luteum upon the interoestrous tube. This particular tube had had previously three separate doses (1 cc. of a 1:10 dilution) of corpus luteum. After each dose the Locke solution chamber was washed out with Locke solution following which tracings were made to determine whether the

tube was capable of resuming the regular rhythmical contractions of interoestrus. In every case it was found that after a short period tubal tracings were similar to those of the interval before addition of the extract. In the figure it is seen that 1 cc. of a physiological saline solution of the ash prepared by charring 1 cc. of the potent corpus luteum extract has little if any effect upon the tube. At a later stage 1 cc. of the extract shows a marked action. After washing out with Locke solution (for the fourth time) it is seen that the tube returns to the natural order of contraction.

Discussion. Notwithstanding the pioneer work of Knauser (5), Burger and Mandl (6) and others who showed that atrophy of the vagina and uterus rapidly follows removal of the ovaries, whereas these organs maintain their normal status if the ovary is transplanted to some other part of the body, little progress has been made since their work to indicate the portion of the ovary responsible for the continued maintenance of the integrity of the uterus and vagina. Much has been written upon the reproductive cycle and the literature is too voluminous to attempt a review of the contributions. In the recent review, "Oestrus, Ovulation and Menstruation," Corner (7) has summarized and brought up to date the knowledge of the entire field. It suffices to say, however, that at present there is misunderstanding and confusion among workers as to the relative importance of the different components of the ovary in the elaboration of internal secretion, such properties being attributed by some workers to the corpus luteum, by others to the follicles and by still others to the interstitial cells of the ovary. With few exceptions, however, the literature bears little evidence of the actual isolation of physiologically active substances from the ovary. In the work of Allen and Doisy (8) substantial progress seems to have been made. These workers have shown that their special extract prepared from the follicles is responsible for the oestrous phenomenon in rats.

Other workers among whom are Bell and Hick (9), Ott and Scott (10) and Stickel (11) have studied the effect of corpus luteum and ovarian preparations upon the excised uterus. Later Matsumoto and Macht (12) studied the effect of similar substances upon the uterus, tube, vas deferens, seminal vesicles and the bladder of various animals and describe a selective action of corpus luteum, similar to that of adrenalin, upon the vas deferens of the rat.

In connection with the present study it might be said that the knowledge of the cyclic variations in the type of spontaneous contractions of uterine and tubal musculature is of recent origin. Blair (13), working with the uteri of the albino rat and Keye (4) with uteri of the domestic pig have demonstrated the presence of muscle contraction variations, those at oestrus differing from those of the interoestrous or quiescent period of the

cycle. The writer (14) first showed that the Fallopian tube of the domestic pig undergoes cyclic variations in spontaneous contraction. It was found that the contractions at oestrus consist of rapid undulations of 10 to 15 per minute, while those of the interoestrous period are characterized by slow rhythymical contractions (4 to 6 per minute) of uniform amplitude. Following this work Seckinger and Corner (15) demonstrated exactly parallel phenomena in a species of monkey, the Macacus Rhesus. More recently Seckinger and Synder (16) studied tubal contractions in the human and found cyclic variations which correspond strikingly to those found in the domestic pig and the monkey. In species so widely separated as the domestic pig, monkey, and the human the contractions during the interoestrous interval are of the nature of slow rhythmical contractions of uniform amplitude (4 to 8 per minute) while those at oestrus or ovulation consist of more rapid undulatory contractions (10 to 15 per minute). To definitely explain the significance of these recent contributions is beyond our knowledge at the present. The exact time of the change of the muscle contractions, however, is so closely associated with the gross and histological changes occurring in the ovary, tube and uterus, that the possibility of hormone influence is most suggestive.

An analysis of the experiments described in the present paper reveals several interesting facts. In the first place it is seen that the tube responds actively to commercial preparations of corpus luteum. This is not the case, however, with all preparations, for of the commerical extracts of corpus luteum tested, only two produced the type of contraction shown in figures 1 and 2, while others showed little or no effect upon either the oestrous or interoestrous tube. In the work of Matsumoto and Macht the literature affords evidence of the influence of certain commercial extracts of corpus luteum upon the vas deferens of the rat. These workers found certain preparations of corpus luteum exert little if any influence upon the vas while others cause marked contraction. It is impossible to explain why certain preparations are active upon the tube while others are inert. These findings would suggest, however, that these differences in potency are due to methods of preparation, involving loss or destruction of active components. While such differences may be due to variations in the quality of glands used it is more probable that they are to be attributed to differences in methods of preparation.

Several observations are outstanding in connection with the series of extracts prepared and tested upon the tube in this laboratory. It was found that the active substance of the corpus luteum is soluble in physiological saline solution. Far more potent extracts are obtained from the dry powdered corpus luteum than from the fresh gland. Removal of proteins with lead acetate in no way lessens the activity of the extract upon the Fallopian tube. While extraction of the powdered corpus luteum with

petroleum ether removes certain fatty substances, the active portion remains intact in the residue. Reëxtraction with acetone after petroleum ether renders the substance inactive (fig. 8) but the active substance is readily recovered from the acetone residue, as will be shown in a subsequent paper. Alcohol and ether extracts after initial extractions with acetone are inert.

Extracts of other portions of the ovary, namely, the mature follicles, the recently ruptured follicles and the stroma, in these series of tests have repeatedly failed to actively influence tubal contractions. From these observations it would appear that active corpus luteum extracts have a selective action upon the tube. Furthermore, the active substance does not injure the musculature of the tube. As many as four separate doses of corpus luteum have been administered at definite intervals to the same ring of tubal muscle. After each response the tube returned to its normal state following washing with Locke solution.

A striking observation is the fact that extracts from the mature follicles, the recently ruptured follicles and the stroma show practically no activity upon either the tube or vas deferens, while the corpus luteum substance active upon the tube produces also a very marked contraction of the vas deferens.

Finally, because of the apparent relationship of the corpus luteum of the living animal to the interoestrous type of contraction, the writer feels that a brief discussion of this relationship and the effects of corpus luteum upon the oestrous and interoestrous tube is in order. In the domestic pig it was found that at the time when the corpus luteum was at its greatest activity in the non-pregnant animal, judging from its size and histological picture, the tubal contractions were of the interoestrous pattern. Tubal contractions of the pregnant pig were also of the interoestrous type. In the monkey the interoestrous pattern was observed at all times except at ovulation. Unfortunately there were no pregnancies in the series of monkeys studied; hence little can be said of the tubal contractions of the pregnant monkey. But in the human it was found without exception in a rather large series of cases covering different stages of pregnancy that tubal contractions were invariably of the interoestrous type. Likewise in non-pregnant women the tubal contractions were those of interoestrus, except at or near the time of ovulation. In the species studied it is interesting to note that *in vivo* the corpus luteum is associated with the contractions of the interoestrous period or definitely apart from the time of ovulation. Whether it is really responsible for the interoestrous pattern the writer is unable to say definitely, but in figure 11 where there is an actual change from the oestrous pattern to that of interoestrus upon addition of an active corpus luteum extract, the possibility of an interoestrous influence on the part of the corpus luteum is most suggestive. Even the activity of

corpus luteum extract upon the interoestrous tube is hardly less suggestive. In this type of contraction, throughout the series of experiments, a change of the contraction *type* has never been observed. The tubal contractions are greatly amplified and at the same time there is a correspondingly slower rate, but the contraction pattern remains essentially that of interoestrus. This observation is not surprising if the fact is borne in mind that the corpus luteum in the living animal is in some way associated with the contraction pattern of the interoestrous period.

SUMMARY

1. Corpus luteum extracts exert a specific action upon the excised Fallopian tube of the domestic pig. After addition of corpus luteum extract the contractions of the interoestrous tube are greatly increased in amplitude, with a corresponding slower rate, but the same general pattern is maintained throughout. The tube of the oestrous period likewise responds actively to corpus luteum extracts but, in contradistinction to the effects obtained upon the interoestrous tube, in a sufficiently large number of cases, there is an actual change from the oestrous type of contraction to that of the interoestrous period.

2. The active corpus luteum substance is soluble in physiological saline solution, and is not removed by defatting with petroleum ether or by deproteinization with lead acetate. The active substance is removed when glands are first extracted with acetone. Physiological saline extracts after such procedure have proved to be inert.

3. Extracts from the mature Graafian follicles, the recently ruptured follicles and the stroma of the ovary exert little if any influence upon the tube.

4. The corpus luteum substance active upon the Fallopian tube likewise causes marked contraction of the vas deferens of the rat.

5. The substance is thermostable, showing little deterioration after boiling for a period of two hours.

The writer wishes to express his appreciation to Mr. A. E. Stickels, of this laboratory, for his interest and coöperation in these experiments and in the preparation of the extracts used.

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FACTORS IN THE CAUSATION OF DIFFERENTIAL BLOOD PRESSURE

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It was shown by L. Hill and M. Flack (14) in 1909 that the systolic pressures in the arm and leg were the same in the normal person in a horizontal position and the same workers in conjunction with W. Holtzmann (15) showed that this was not true in patients with aortic regurgitation, in whom the femoral systolic pressure might be much above that in the brachial. L. Hill and R. A. Rowlands (16) extended these observations, and found in cases of aortic regurgitation differences in favor of the femoral of pressures varying between 22 and 150 mm. of mercury and with an average of 53 mm., and the difference between the arm and leg was sometimes more marked on one side of the body than on the other. In the same paper (16) they also quote figures communicated to them by Lewis obtained on a dog with chronic experimental aortic lesion, where the systolic pressure in the femoral reached 240 mm. and in the brachial only 198; this same experiment on the dog gave a lower diastolic pressure in the femoral than in the brachial, being 58 mm. in the femoral and 74 in the brachial.

This "differential" pressure in favor of the femoral seems to be only an exaggeration of what is quite a common and normal occurrence, which has been observed by many people in animals, so that Hill and his coworkers were inclined to attribute the differences in aortic disease to a better conduction of a pulse wave into the femoral vessels. They also showed that the conduction of such a wave in a schema was greater in a tensely distended artery than in one less distended and consequently concluded that the difference was due to a diminished distensibility of the femoral vessels, as a result of a contraction of the muscular wall. In support of this theory they found that the difference between the arm and the leg might be abolished by the immersion of the lower half of the body in hot water, a process which may be presumed to cause vascular relaxation in the lower limbs. Another fact somewhat in favor of their theory, though not used as an argument by them, is that Lewis's dog with aortic regurgitation presented a lower diastolic pressure in the femoral than in

the brachial and this again is merely an exaggeration of the differences seen in the normal dog, a good example of which may be seen in curves published by O. Frank (10) (figs. 28 and 29 of his paper).

L. Hill and Rowlands (16) also record differences in pressure up to 50 mm. between the arm and leg in occasional cases of arteriosclerosis, but the average difference was only 8 mm.

Since these observations of L. Hill, the phenomenon has been frequently observed clinically in cases of aortic regurgitation and has been described also in other conditions which are accompanied by a large pulse pressure. Thus A. E. Taussig (30) has described a similar differential blood pressure in cases of exophthalmic goiter, in which he found the femoral systolic pressure to be above the brachial by 20 to 57 mm. with an average difference of 37 mm. He found the diastolic pressure to be also raised, but only slightly, varying from 1 to 20 mm. with an average difference of 7.6 mm. Thus the differences were less than in cases of aortic regurgitation and here evidence is also brought forward of a raised diastolic pressure. Taussig in addition made observations on cases of aortic regurgitation and found the systolic pressure raised by 30 to 85 mm. and the diastolic pressure also 1 to 10 mm. higher in the femoral than in the brachial.

Doctor Sailer (26) has independently investigated the blood pressure in cases of exophthalmic goiter finding a differential blood pressure of about the same magnitude, though as a rule the diastolic pressure was lower in the leg than in the arm.

I am indebted to Doctor Sweet (29) for a personal demonstration of records of femoral and carotid blood pressures taken in dogs with a mercury manometer in which the mean femoral pressure was often slightly higher than the carotid, and more noticeably so in dogs with aortic regurgitation.

There seems therefore to be ample evidence of the existence of Hill's phenomenon, and yet very little either for or against his theory of its causation.

I have used a circulation schema in teaching my students the principles of the circulation, and it has proved possible to imitate this so-called differential blood pressure in such a schema and here the apparent cause is the conversion of kinetic energy into stress when the moving fluid meets an obstruction.

A differential pressure may be produced by several methods in animals. The results obtained with the schema will be first described, then those obtained in animals for comparison.

PART I: SCHEMATIC CIRCULATION: *Method.* The type of circulation. schema used has been a rubber bag or large glass bottle containing water hung at a considerable height (1 or 2 m.) above the table so as to supply fluid at a high pressure. This reservoir is connected with the circulation

schema proper by heavy wide bore rubber tubing and joins a tap which opens and allows fluid to flow from the reservoir into the schematic aorta at intervals. The aorta and large vessels have been represented by soft distensible rubber tubing, most of the records being obtained with black tubing of 6 mm. bore with a wall 1 mm. thick. The resistance in the arterioles and capillaries has been represented by glass tubes of about 20 mm. bore packed at their central end with glass wool and more peripherally with cotton. When the tap is open, fluid passes into the distensible tube at a pressure regulated by the height of the reservoir, which therefore determines the systolic pressure, and when it closes, the distended tube empties itself through the peripheral resistance.

This type of schema was one which was used by the writer in a primitive form during the war (2) and has been improved by the addition of the cotton resistance, instead of the use of fine tubes, so that now the schema offers a capillary resistance, which will allow fluid to pass in inverse proportion to its viscosity, which proved not to be the case in the older type.

Most of the records have been made with a schema of this type with a rotatory metal tap designed by Lodholz (23) and used in this laboratory for teaching students for many years. This design is specially arranged to reproduce the conditions of aortic regurgitation. Such a rotary tap gives a constant ratio of systole to cycle (varying according to the tap used between $\frac{3}{10}$ and $\frac{4}{10}$) at whatever rate it is driven, and its opening and shutting are probably relatively slow as compared with the movements of the aortic valves.

Lodholz's valve has a groove on the rotatory portion of the tap which connects the schematic aorta during diastole with a small cock; by opening this the degree of regurgitation can be regulated. For mechanical reasons it is impossible to make the aortic regurgitation commence immediately the inflow of fluid into the aortic tube ceases, but only after a short interval, so that the resultant curves are not exactly those of aortic regurgitation in an animal.

The general arrangement of the circulation model may be seen in figure 1, which represents an experiment (to be referred to again later) where the arrangement was the most complicated employed. In this experiment the character of the tubing was altered in parts of the system, tubing of 3.5 mm. bore and of 2.0 mm. thickness in the wall being used; this thicker tubing is indicated in the diagram by thicker lines. Variations in the character of the wall were experimented with in this way, to test L. Hill's suggestion, but as will be seen later, these variations in the character of the wall, while producing definite differences, were not found to be essential.

In the schema as arranged in figure 1, the tube *I* represented the innominate, carotid and subclavian, the record from *C* being taken to correspond with the carotid. In most of the experiments the length from *A* (representing the aortic arch) to *C* was much shorter and unbranched being about 5 cm. in length instead of 20 cm. as in this case. The section *Th A* represented the thoracic aorta, and the right angled branch to *R 3* vessels leaving the aorta at right angles, such as the renal vessels. *A. A.* represented the abdominal aorta, *B* its bifurcation into the common iliacs and the record from *F* was taken to correspond with the femoral. The branching of the "innominate" vessel in this case is obviously unlike that seen in an animal, but it was purposely so arranged in this particular experiment, so as to reproduce in the "carotid" system an arrangement of branches more closely resembling in size those of the "femoral" system. The whole schematic system was kept horizontal throughout all the experiments.

The records of pressure changes were taken with a Hürthle manometer or with a membrane manometer of Porter's pattern as made by the Harvard Instrument Company, and the two points of the circulatory system which were being compared were connected with the same manometer by means of a 3-way stopcock. The manometers were connected with the recording points with equal lengths of glass tubing of 5 mm. bore joined to the manometer, side tubes, and stopcock by short lengths of rubber pressure tub-

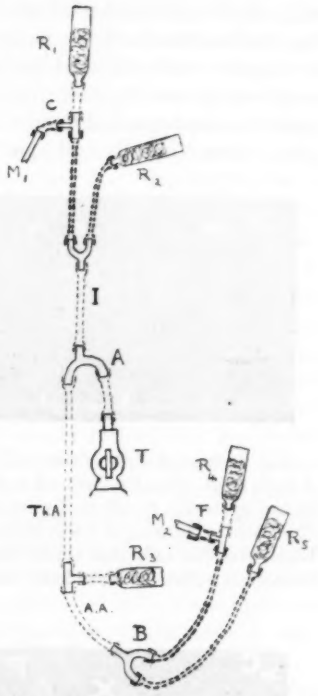

Fig. 1. Sample arrangement of schema (scale $\frac{1}{8}$). *T*, Lodholz valve; *A*, aortic arch; *I*, innominate system; *C*, recording point corresponding to carotid; *Th A*, thoracic aorta; *A A*, abdominal aorta; *B*, bifurcation into iliacs; *F*, recording point representing femoral; *R1* to *5*, resistances representing the arterioles; *M1* and *M2*, tubes leading to 3-way stopcock and so to manometer. Solid lines represent metal or glass tubing. Dotted lines rubber tubing—thin, 1 mm. wall 6 mm. bore; thick, 2 mm. wall 3.5 mm. bore. Lengths: *T* to *A*, 4 cm.; *I*, 9 cm.; *I* to *C*, 11 cm.; *C* to *R1*, 3 cm.; *I* to *R2*, 9 cm.; *Th A*, 21 cm.; *A A*, 8 cm.; tube to *R3*, 3 cm.; *B* to *F*, 16 cm.; *F* to *R4*, 1 cm.; *B* to *R5*, 19 cm.

ing. Such a method is obviously only roughly accurate, since the recording mechanism is by no means free from inertia and momentum, but such errors were reduced by using a rubber membrane that gave very small excursions for large changes in pressure (since no attempt was made to read small differences accurately) and also by using a slow pulse rate (usually 56 per minute), allowing comparatively slow

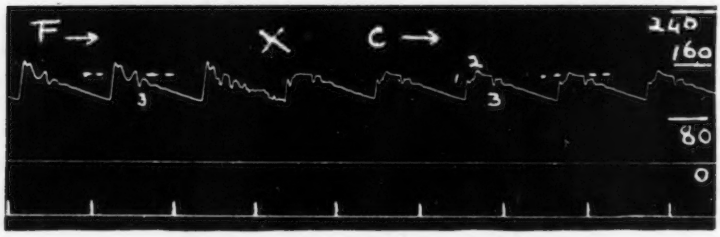

Fig. 2. Record with schema, all 6 mm. soft tubing. No regurgitation. Lengths: *A* to *C*, 3 cm.; *C* to *R1*, 2 cm.; *T* to *F*, 60 cm.; *F* to *R4*, 3 cm.; *B* to *R5*, about 7 cm. *1*, Initial upstroke; *2*, reflected wave on plateau; *3*, dicrotic notch. *F*, femoral record; *C*, carotid; *x*, turning of 2-way stopcock. Scale = pressure in millimeters Hg. Dotted horizontal line indicates actual pressure available from reservoir. Time intervals in seconds. (Record reproduced about 3/2.)

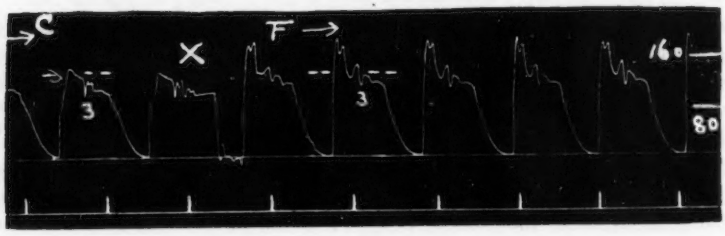

Fig. 3. Record as in figure 2 but with maximal regurgitation

primary changes in pressure. Since the two records were compared on the same manometer, the fling of the lever and other errors would remain fairly constant, and it is only from differences between the two records that conclusions are here drawn. That the records followed fairly truly the changes in pressure in the tube, with little distortion from the vibration character of the recording system, was indicated by the way in which the secondary waves could be modified by varying the length, etc. of the schematic aorta, while they were little affected by changing the recording system.

Such a system gives pressure curves approximating those found in an artery, as is shown by figure 2, which reproduces curves obtained without regurgitation from a schema arranged much like that of figure 1, but consisting entirely of thin walled tubing, with a short 5 cm. unbranched carotid and with a length from tap to the recording point *F* of 60 cm. The record shows the femoral pulsation *F* and carotid pulsation *C*, the change being made in the center of the records at *X*. In addition there was another oblique branch leaving the aorta between the branch to *R 3* and *B*, which was intended to represent the mesenteric vessels. The record obtained from *C* will be seen to resemble closely the photographic record obtained from a rabbit's carotid by C. Tigerstedt (31) while the record from the point *F* shows only slightly greater differences from his femoral records. Several of Tigerstedt's curves are reproduced here for comparison (fig. 18). From the point of view of experimental errors in the recording apparatus it should be noted in figure 2 that the highest point in the carotid pressure (marked *2*) occurs not at the beginning where upfling of the lever might exaggerate it but later, where errors from momentum will be minimal, and that even in the femoral record the height of the systolic pressure is not exaggerated on the record to any extent, since the second wave on the initial plateau is nearly as high as the first upstroke. It is noticeable that the femoral record on the schema gives a slightly higher systolic pressure and about the same or very slightly lower diastolic pressure as compared with the carotid, corresponding with Frank's observations on animals.

A further point must be noted. It has been stated that in this form of schema the systolic pressure is limited by the height of the water reservoir and can be altered by changing its position, but it is noticeable that the record shows higher pressures than those obtainable from the height of the bag. If, that is to say, all the points of outflow are blocked and the tap is left open, so that the manometer reaches a stationary position at the maximum pressure, this is found to correspond in the case of the "carotid" pulse wave with the plateau following the wave marked *2*, so that part of wave 2 lies above the level of the maximum available pressure and yet occurs at a time when upfling of the lever cannot distort the curve. Similarly the femoral curve reached above this maximum pressure but at a point where over-fling of the lever might account for the result, if it were not for the plateau like character of this part of the curve with a second wave added to it. There can be no doubt therefore that the pressure does rise above the maximal pressure available from the reservoir. The details of the curves are important, for it is these waves that are particularly exaggerated by aortic regurgitation, thus accentuating the differences between the "carotid" and "femoral."

In order to exclude completely artefacts through errors in the recording instruments a few photographic records have also been made. These records were obtained by the use of T tubes in the same position as those connecting with *M1* and *M2* in figure 1, but the side tube was of 11 mm. diameter and 3 to 4 cm. long and its end was covered with rubber dam (compare fig. 10). The tambours thus made were connected by rubber tubing and air transmission to Frank capsules. Curves produced in this way, with the schema arrangement the same as for figure 2, are reproduced in figures 19 and 20 and it will be seen that the general character of the cuves (which still strikingly resemble those of Tigerstedt), and the differences between the carotid and femoral remain the same.[1] In the photographic curves with air transmission in this way the character of the curves is no doubt very accurate, but the actual pressures can rarely be read closer than to the nearest 10 mm. Hg. The two points cannot be connected to the same tambour without introducing the error of a long column of fluid in the recording system, so that recording on two such separate systems does not increase the accuracy of comparison of relative pressures. Consequently photographic methods have only been used to check the other method, and to determine the exact character of the waves rather than their exact pressure.

Differential pressure in branched system. It has already been pointed out that in this schema of branching tubes, the systolic pressure recorded in the femoral is slightly higher than that recorded in the carotid, and that, in both, the recorded pressure may be higher than that available from the reservoir. If the conditions of aortic regurgitation be now imitated by opening the small cock these differences are much exaggerated as as may be seen by reference to figure 3, obtained mmediately after figure 2 but with this small cock opened to its maximum. The recorded pressure now rises very much above that of the reservior.

Differential pressure in single tube. A simpler example of the same phenomenon may be obtained in a single unbranched elastic tube. Figure 4 gives curves obtained in a single tube of 6 mm. diameter varying in length from 125 to 53 cm. Curve *a* was obtained without, and *b* with, an aortic leak in a tube of 125 cm. length; curves *c* and *d* with tubing of 74 and 53 cm. length with regurgitation. It will be seen that the single tube gives curves closely resembling those of the branched system (though a stage further removed from the character of a true pulse curve), and that the aortic leak still exaggerates previous existing waves. It is also clear that the difference in pressure between the two ends is slight

[1] It may be noted that the apparent marked dicrotic wave in the femoral in figure 20 *a* commences before the dicrotic wave and is really a reflected wave. It therefore only imitates the dicrotic of the femoral pulse if this is a reflected wave as supposed by Frank (10).

with a very long tube, is increased as it is shortened, and then is decreased again as the tube is still further shortened. There is therefore an optimal length at which this difference is most marked, and this optimal length is found to be greater the narrower the tube and the lower its distensibility.

In table 1 are given the actual pressure values obtained in the experiments partly reproduced in the figures. It will be seen that in the above

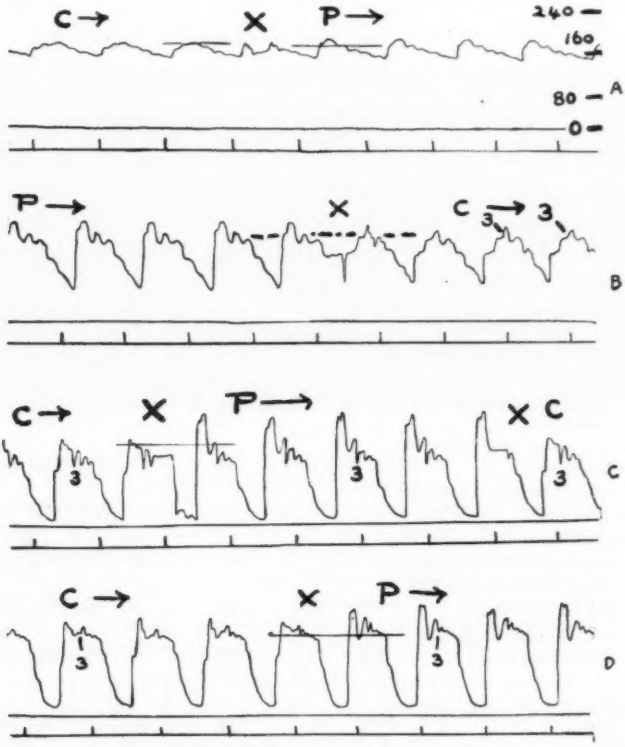

Fig. 4. *a*, Record with single tube 6 mm., bore 1 mm. wall and 125 cm. long without regurgitation. *C*, record from central end close to valve. *P*, record from peripheral end close to schematic arteriole. *b*, as before with regurgitation; *c*, as in *b* but with tubing 74 cm. long; *d*, as in *b* and *c*, but with tubing 53 cm. long. Other lettering, etc., as before. Record redrawn for greater clearness. (Reproduced about 1/1.)

experiment the degree the pressure at the peripheral end of the tube rises above that of the reservoir (obtained by adding the two last columns in the table) varies with the length of the tube, there being an optimal value. The degree the pressure at the central end rises varies similarly, but the optimal value is not the same. At the central end of the tube

it will be noticed that the highest point of the curve comes late with a reflected wave (the pulse wave velocity was usually about 12 meters per second) and an anacrotic type of pulse in *b*, and is seen much earlier with a katacrotic type of pulse in *d*.

TABLE 1

Schema values

NUMBER OF FIGURE	CONDITION	LENGTH OF TUBING *A* TO *C*		LENGTH *A* TO *F* OR *P**		RESERVOIR PRESSURE	SYSTOLIC PRESSURE AT *C*	SYSTOLIC PRESSURE AT *F* OR *P*	DIFFERENCE BETWEEN	
		Soft	Harder	Soft	Harder				*C* and reservoir pressures	*C* and *F* or *P* pressure
		cm.	*cm.*			*mm. Hg*				
2	Multiple tubes normal	5	Nil	60	Nil	145	150	163	5	13
3	Multiple tube regurgitation maximal	5	Nil	60	Nil	145	152	192	7	40
4a	Single tube normal			125		172	175	185	3	10
4b	Single tube regurgitation submaximal			125		172	190	200	18	10
4c	Single tube regurgitation submaximal			74		164	170	235	6	65
4d	Single tube regurgitation submaximal			53		164	180	225	16	45
4e	Single tube regurgitation submaximal			33		164	172	184	8	12
7	Single tube normal and regurgitation very hard				48	151	167	164	16	−3
8a	Multiple tubes regurgitation submaximal	9	11	29	16	163	180	210	17	30
8b	Multiple tubes regurgitation submaximal	3	11	29	16	163	175	215	12	40
9a	Multiple tubes regurgitation	3	11	29 (very soft)	16	152	162	160	10	−2
9b	Multiple tubes regurgitation	3	11	21 (very soft) +8 ordinary	16	167	175	195	8	20

**F* has been employed in the figures to mark a peripheral record in branching tubes resembling the circulatory system and *P* where the peripheral record is obtained from a single unbranched tube.

It is clear that a differential pressure, similar to that of the circulation, may be seen in a simple elastic tube distended rhythmically, and that the condition is intimately associated with reflected waves. Further

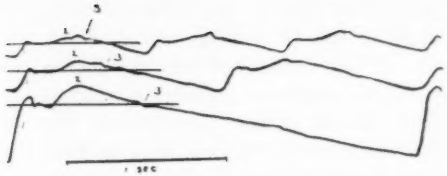

Fig. 5

Fig. 7

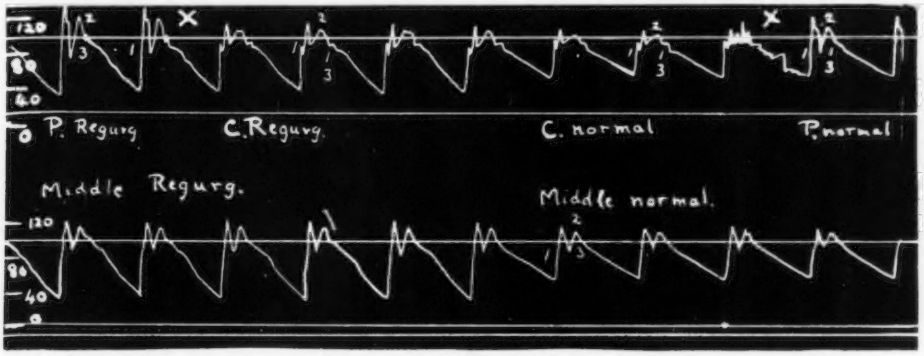

Fig. 6

Fig. 5. Effect of pulse rate on type of curve in schema. Reproduced 2/5. Single tube about 75 cm. in length. Record from central end. Tap operated by cam. Change of pulse rate from 23 to 46 and 72 from below upwards. Line through systole gives pressure from reservoir, namely, 150 cm. of water. Primary wave, reflected wave and dicrotic marked *1*, *2* and *3*. (Redrawn to obtain superposition.)

Fig. 6. Record with 2 manometers. Lower manometer recording pressures at center of rubber tubing. Upper manometer connected with 3-way stopcock and so with central or peripheral end of tube (*C* and *P*). Curves with regurgitation to left, without to right. Horizontal lines give level of reservoir pressure. Valve cut so that $\frac{\text{systole}}{\text{cycle}} = \frac{1}{3}$ and pulse rate only 37 (i.e., systole extra long). Relative low arteriole resistance. Length from tap *T* to recording point *C*, 3 cm.; *C* to tube connecting with lower manometer 27 cm.; from here to bifurcation 17 cm. and from bifurcation one tube 3.5 mm. bore 2 mm. wall 19 cm. long to peripheral resistance and one ordinary thin wall 6 mm. tubing 18 cm. long to recording point *P*; *P* to peripheral resistance 1 cm. Lettering as before. (Reproduced 1/1.)

Fig. 7. Record with a single tube of old very hard rubber tubing 6 mm. bore and 2 mm. wall of 48 cm. length. With regurgitation records from both peripheral and central ends, and record from central end without regurgitation. (Reproduced 1/2.)

evidence of the importance of reflected waves may be seen in figure 5. In this case records were taken from the central end of a single unbranched tube of pure gum rubber of about 75 cm. length without regurgitation, but the tap used was one with a cam drive, allowing a more sudden opening

and shutting, and the relative durations of systole and diastole were varied with changes in pulse rate more or less in the manner in which these factors would alter in an animal. It will be noticed that the degree that the secondary wave *2* rises above the reservoir pressure is related to the pulse pressure, and here varies in the inverse direction to the pulse rate. The secondary wave *2* also remains at a uniform time interval after the primary wave, as long as the length of tube is kept constant. A mere change in pulse rate may completely alter the general appearance of the pulse curve, by causing the reflected wave to return at a different part of the cycle, so that the change in pulse rate could account for many of the differences to be seen between the lowest and highest of the carotid curves of Tigerstedt (31) reproduced in figure 18 *a*. Similar changes with a smaller change in pulse rate may be seen in comparing figures 19 *b* and 20 *a*, where differences in the femoral curve may be noticed and compared with those of Tigerstedt reproduced in 18 *b*. It is therefore at least possible that this dominance of reflected waves is not peculiar to the schema.

Probable causation in the schema. The fact that the recorded pressure rises considerably above that available from the reservoir must indicate a fusion of primary and secondary waves temporarily raising the pressure. Evidence that reflected waves are partly concerned has already been brought forward. Another possible cause would be the conduction of waves at different velocities so that one might overtake the other, a possibility in the circulation itself that has already been considered by Bramwell and A. V. Hill (4). That this type of conduction might be anticipated was realized by Grunmach (12), who tried to duplicate it in a schema by using rubber tubing covered with linen to give a variable elasticity. With the idea that Grunmach's experiments might supply the clue to the causation of differential pressure, the first experiments were made using tubing covered in this way, but the differential pressure was obtained whether the covered rubber or plain rubber was used. Grunmach also showed that the modulus of elasticity of rubber was fairly constant over a wide range of pressures, so that the conditions for the setting up of the "breaker formation" described by the above authors are probably absent in the schema.

There is another factor present intimately associated with the reflection of waves, namely, the transformation of kinetic energy into stress. Bernoulli's theorem expresses the fact that, apart from loss by friction, the sum of the potential energy, pressure energy, and kinetic energy will remain constant. At the central end of the tube the velocity is intermittent, being zero in diastole, and even in systole it is not constant but must show large and rapid variations. At the peripheral end of the tube on the other hand the outflow is continuous with relatively much

smaller variations. Consequently during systole a much greater proportion of the energy must be in the kinetic form in the central end of the tube than in the peripheral, and the stress should therefore be greater at the latter point. If this were an important factor then the pressure at the end of the tube should always rise higher than that at any other point. Figure 6 shows an experiment in which a continuous record was obtained from the middle of a branched system, while another manometer gave records alternately from the central and peripheral ends. It will be seen that both as regards the level of the systolic pressure and the character of the curve, the record taken from the middle of the tube shows an intermediate stage. In this experiment the total length of the tube was about that giving the optimal differential pressure, and it might be objected that the pressure obtained from the middle of the tube would necessarily be lower, since it was not at the point of optimale ffect. If, however, a length of unbranched tube be taken such that the length is about double that at which the pressure difference between the two ends is greatest, and a record be then taken from the middle of the tube, it is still found that the pressure developed at the end is greater than that at the middle, although the latter record is being taken from the optimal length of the tube. Under these circumstances however the difference in pressure reached between the end and the middle is not very great, nor does the record from the middle necessarily keep an intermediate position in the character of its waves.

So far the whole phenomenon therefore seems most readily explicable in the schema mainly as the result of conversion of kinetic energy into stress, and the unequal distribution of the resultant pressures through reflected waves, and, if this is so, it should vary in degree with the square of the velocity attained, with the mass involved, with the degree of transformation into stress, and with the irregularity of its distribution in the system. It will be seen that these factors are not easily altered singly in this schema, and a mathematical treatment, though perhaps possible, would be extremely complicated, so that this explanation has been put to the test by varying the conditions of the experiment, and seeing if the observed results are in agreement with this theory.

KINETIC ENERGY THEORY TESTED. *Changes in velocity.* These should prove particularly effective since kinetic energy is represented by half the product of the mass and the square of the velocity, and it is therefore clear that it is the greatest velocity reached that is the most important rather than the average velocity, since a high velocity attained by a relatively small mass will be more effective than a proportionately lower velocity in a larger mass. The velocity with which the fluid flows into the schema tube when the tap is open will obviously depend on the difference of pressure between the two, and will therefore be increased, if the pressure at the end of diastole in the tubing be reduced by any means.

A simple means of lowering the diastolic pressure is to slow the pulse rate, so allowing a longer period for the pressure to fall. Such a procedure increases the primary wave, and also the secondary waves, the height the pressure rises above that of the reservoir (see fig. 5), and also increases the differential pressure (compare figs. 19 *b* and 20 *a*).

Other methods of varying the velocity are increasing the height of the reservoir, or lowering the peripheral resistance, so as to increase the total flow. Both increase the differential pressure, but the latter only if kept within certain limits, since if the resistance is too low, the peripheral slowing of the stream during systole will be absent. But the greatest velocity changes probably occur when fluid is drained from the central end of the tube during diastole through the imitation aortic leak, when the diastolic pressure may be reduced to zero, if desired. These several points may be seen clearly by a detailed study of the experiment represented by figures 19 and 20.

In figure 19 *a* the bag was set to give a pressure of 108 mm. Hg and the recorded pressure in the "carotid" was 95 mm., in the femoral 140, giving a difference of 45 mm. The diastolic pressures fell in each to about 20 mm. In figure 19 *b* everything was as before except that the bag was raised to give a pressure of 140 mm. thus causing a greater wave and more rapid ejection of fluid at each beat. The carotid pressure was raised to 140, the femoral to 195, a difference of 55. The diastolic pressure was still in both about 20 mm. Thus changing the pulse pressure by producing a greater and more rapid ouflow caused a greater differential blood pressure. In figure 20 *a* everything was left as in 19 *b* except that the rotating tap was driven at a slightly faster rate. The pulse rate was now 45 as compared to 38 previously. The diastolic pressure does not fall as low as previously since the period during which the fall occurs is curtailed (the diastolic pressure being here partly a function of pulse rate just as it is in man, a point too often neglected) consequently with a less initial difference in pressure the fluid is ejected less rapidly and also in less amount. With the diminished output per beat and smaller pulse pressure the "femoral" pressure now only reaches 180, the "carotid" reaching 140 as before so that now the difference is reduced to 40 mm. The diastolic pressure was about 40 mm. With the introduction of a considerable aortic regurgitation, as in figure 20 *b*, however the pulse pressure was enormously increased, the diastolic pressure being reduced to zero, the inflow of fluid in systole must have been rapid and large in quantity and the recorded pressure in the "femoral" rose to 240, that in the carotid to 160, showing a difference of 80. Throughout the experiment the peripheral resistance was low, the records without regurgitation being intended to imitate the differential pressure observed in exophthalmic goiter.

This example not only illustrates the above mentioned points, but also suggests that a big pulse pressure produced by regurgitation is more effective in causing a differential pressure than is one resulting from a low peripheral resistance. Other experiments confirm this. This is entirely parallel with clinical findings, and is also readily explained by the theory, since with a low peripheral resistance the velocities may be high and show big fluctuations, but the slowing of the velocity of the fluid as it meets the peripheral resistance will be less complete, and the degree of transformation into pressure consequently less.

Changes in velocity may also be produced by changing the size or character of the tubing. If the fluid entering at each systole is kept constant, the velocity reached must be higher the smaller the diameter of the tubing, and it is found that narrow tubing is more effective than that of larger diameter. Again, if the tube is relatively non-distensible, the pulse pressure may be great, and the diastolic pressure very low, but this pressure is rapidly raised by a small entrance of fluid, and, after this, fluid can only enter at the rate at which it leaves the periphery. Even though the pulse pressure is great, under these circumstances the velocities are low and the differential pressure is not in evidence. Figure 7 provides an example of this obtained with heavy tubing of about 2 mm. wall, which had hardened with age and become very little distensible, There is little difference to be noticed between the records obtained from the central and peripheral ends. As the result of this factor interacting with others, it is found that there is an optimal distensibility at which the differential pressure is most marked, since changes in distensibility not only affect the velocity attained, but also the distribution of any stress developed, as will be seen later.

Variations in mass. These must also be important, and evidence of this has been already produced, since it has been shown that there is an optimal length for a single tube, at which the pressure at any point rises most above the reservoir pressure. The longer the tube the greater the mass and therefore presumably the greater the kinetic energy, but if the tube be sufficiently long the entering fluid will be easily accommodated and the inertia of the fluid contents may prove an adequate resistance, so that the whole column is not set equally in motion and the pressure resulting from transformation of kinetic energy is more widely distributed.

It is not surprising therefore that there is an optimal length of tubing, and that this optimal length should be greater, the narrower and less distensible the tubing, which is found to be the case.

But the effect of relative mass may be more clearly seen in an experiment with branching tubes. Such an experiment is reproduced in figure 8. The curves of figure 8 *a* were obtained with the schema as illustrated in figure 1 and those of 8 *b* after shortening the tubing between *A* and *C*

by replacing *I* with tubing 3 cm. in length. By this diminution of the carotid system by 6 cm., the differential pressure is much exaggerated, showing a 10 mm. increase. This increase has occurred through both a lowering of the "carotid" pressure and a raising of the "femoral." It will be seen that the degree that the carotid pressure rises above that of the reservoir varies with the length of tubing between it and the schematic aorta, and that this alteration has also resulted in a change in the femoral pressure. Here again the results are entirely in accord with the theory; the lessening of the pressure in the carotid system should follow the decrease in the mass of fluid in these branches, while the decrease in the volume of the elastic system in the carotid branches should leave a larger amount of kinetic energy available for transformation into stress in the

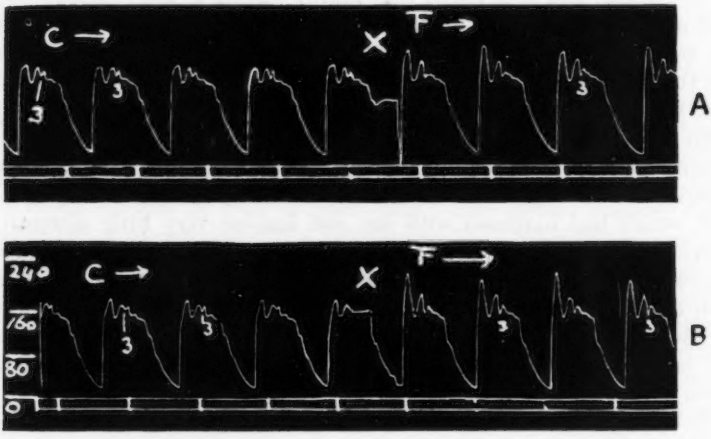

Fig. 8. *a*, Record with schema arranged as in figure 1. (Reproduced 3/2.) Marking as in figure 2. *b*, Record after shortening *I* from 9 cm. to 3 cm. length.

rest of the system. The experiment shown in figure 8 demonstrates that on this kinetic theory a differential pressure can be expected in a branching system, according to the variations in mass of the branches, and that a higher pressure in the femoral vessels, which form a direct continuation of the aorta, might be predicted. Any such stress that is developed cannot be equally effective throughout the system.

Variations in transformation and distribution. In the schema there is never any absolute check to the velocity of the fluid, which has a continuous outflow. The relative check occurs owing to the elasticity of the reservoir allowing a rapid inflow during systole, this systolic inflow being equal to the peripheral outflow in the whole cycle. The shorter the duration of systole in proportion to diastole, the greater must be these variations

in velocity. This effect and also the variations, not only in velocity developed, but also in degree of transformation of energy produced by lowered peripheral resistance have already received some mention and need not be further elaborated.

Variations in the distensibility of the wall are also of extreme importance, since the more distensible the wall the more gradual will be the slowing of the velocity, parts of the column being brought to rest later than others, and the pressure produced will not reach so great a height. The whole phenomenon is really that of water-hammer in a very elastic system, and must obey general laws (11) so that any distensibility will tend to diminish the effect produced. The complicating factor of such a system is that only if the system is elastic can high velocities be acquired by the fluid, while this very elasticity tends to diminish the water-hammer effect by making the slowing of the motion more gradual. Some energy is also lost as heat in the stretching of the walls, but for physiological purposes this can probably be neglected. It has already been shown that the differential pressure disappears if the distensibility is too low—it is not surprising that it can also disappear if this is too great, and that there is an optimal distensibility for the production of the effect, just as there is an optimal length.

An experiment illustrating this is shown in figure 9. The record of figure 9 *a* was obtained in an arrangement of tubes similar to that which was used for figure 8 *b* except that the tubing marked *Th. A.* and *A.A.* in figure 1 was replaced by tubing which had been softened and increased in diameter by being soaked in xylol and dried. It had a bore of 7.5 mm., a wall of about 1 mm., and was extremely distensible. It will be noticed that the usual differential pressure is no longer seen, but that on the other hand it is even slightly reversed. On replacing the length *A.A.* with normal tubing, leaving the *Th. A.* section as before, an intermediate stage was obtained—shown in figure 9 *b*—in which some differential pressure of the normal type is again present, though much less than that of figure 8 *b*.

The greatest development of differential pressure might, therefore, be anticipated if the central end of the reservoir was distensible, affording little resistance to the rapid inflow of fluid, while the peripheral parts were much more rigid. This would appear to be the condition in the circulatory system itself (18). To test this out a schema was arranged as shown in figure 10, and the photographic records shown in figure 21 were obtained. The magnifications of the different tambours were unequal and only approximate comparisons can be made. It will be seen that with regurgitation and the reservoir delivering water at 112 mm. Hg pressure, the relative inelastic branch consisting mainly of glass gave a pressure reaching to about 180 mm., while the peripheral elastic

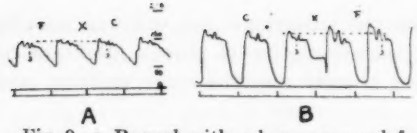

Fig. 9. *a*, Record with schema as used for 4*b* but with very soft tubing 7.5 mm. bore for *Th A* and *A A*. *b*, Record as in *a*, but with *Th A* replaced by 6 mm. tubing. (Reproduced 1/2.)

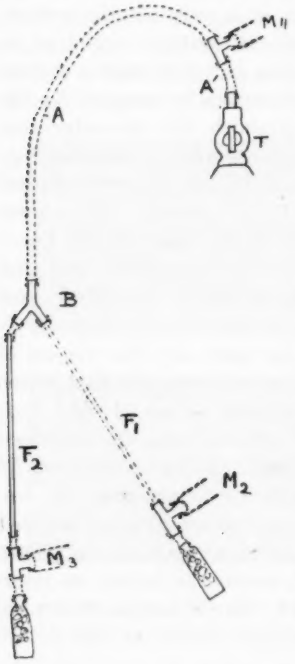

Fig. 10. Arrangement of schema to show effect of variations in elasticity as used to produce record reproduced in figure 21. (Scale 1/8.) *T*, Lodholz valve; M_1, T-tube with tambour covering side tube this being connected by air transmission to Frank capsule giving record *C* of figure 22. *A*, soft rubber tubing 6 mm. bore, 50 cm. long. *A'* ditto 3.5 cm. long. *B*, glass Y tube. F_1, soft tube 4 mm. bore, 1 mm. wall, 25 cm. long. F_2, glass tube 4 mm. bore, 25 cm. long. M_2 and M_3 tambours giving records from F_1 and F_2.

branch only reached 150 mm. The central end gave a delayed rise of pressure to about 160, somewhat above that of the elastic peripheral branch, probably through the fusion of two reflected waves. Without regurgitation (with lower velocities) the central and peripheral elastic ends both gave about 112 mm. while the less distensible glass branch gave 120 mm. It is clear that the hard glass tube accentuated the condition, demonstrating the effectiveness of the factor suggested by L. Hill and his co-workers, but this is not essential to the occurrence of the phenomenon.

Many of the factors that have been discussed have introduced unevenness of distribution of pressure, which must obviously be also concerned. An elastic system does not transmit a wave without loss, and any pressure developed at one end of a tube will be diminished during its transmission to the other end. But more important than this is the time taken in transit. This time is in the circulation in man quite considerable (3), and it is certain that a wave originating in the lower part of the leg cannot be reflected back to the aorta during the time of the heart's expulsion period; if however it returns during the diastolic fall of pressure it can only somewhat delay the fall and cannot produce any great rise. The varying type of curve which results by the way the secondary wave fuses with other waves may be seen in the examples shown in figure 4 where the duration of the waves is altered by changes in the length of the tube, and in figure 5 where the duration of the heart cycle is varied. The importance of this factor in varying the degree of differential pressure developed is obvious.

One may therefore conclude that in the schema the differential pressure seen under varying conditions receives an adequate explanation on the assumption that it arises from

the conversion of kinetic energy into stress, which is unequally distributed through the system, and which varies in a branched system with the relative mass of fluid in any part of the system. Variations in the character of the wall may influence its production but are not essential to it.

PART II: ANIMAL EXPERIMENTS. In comparing records taken from animals with those of the schema, attention must first be drawn to certain differences in the conditions. The resistance to outflow in the schema is high in comparison with an animal, so that the velocities reached are probably lower. On the other hand this is to some extent off-set by a more sudden peripheral slowing, and by the greater reflection of simple waves as compared with the complicated multiple reflections which must occur in the circulatory system. But in the schema experiments pressures have been recorded through side tubes without interruption of the flow, and in the animal experiments a cannula has been tied into the vessel, thus obstructing the flow completely, though flow continues to occur through the branches above this. In the measurement of arterial pressure in man there is similarly a complete obstruction of the flow, and the conditions in both animal and human observations are then optimal for the conversion of kinetic energy into stress in the vessel used.

Method. The animals were dogs under morphine ether anesthesia and in many cases relatively large animals have been used. Since the schema demonstrated the importance of mass, it seemed advisable to work with animals as near to the size of man as was practicable. A similar method of recording was used, and the intervening stopcock also allowed the two vessels to be directly connected to each other, when it was found that blood often passed quite rapidly from one large vessel to the other, thus demonstrating the reality of a difference in mean pressure.

Records were usually first taken from the etherised animal, and then an aortic valve lesion was produced by pushing a mushroom-shaped metal stylet down the right carotid until it caught in the valve and broke it. In a few experiments a hook was used and the valve torn away, when the observed differential pressure was greater, than when the damage was less. In some animals either before or after the above procedure the spinal cord was divided at the level of the 2nd cervical vertebra and the brain above this was pithed. In other experiments the peripheral resistance was varied by adrenalin injection, splanchnic stimulation, obstruction of the one femoral artery (while recording from the other) or by the introduction of an arterio-venous anastomosis by means of a simple U glass tube, modelled on that used by Heymans (13), such an anastomosis being sometimes made in the neck and sometimes in the leg.

I am indebted to Sir Thomas Lewis for an advanced report of his experiments which led to my own experiments with these anastomoses.

One experiment is also quoted in which an aortic valve lesion was made aseptically and the pressures recorded some months later. No differences are detectable between this result and those of acute experiments.

Results obtained. It has already been pointed out that the systolic pressure in the femoral artery in the normal dog is above that in the carotid. My experimental results in normal animals always showed such a relation. Probably there is little difference in mean pressure between the two vessels, since the diastolic pressure falls lower usually in the femoral. Occasionally the two vessels have been left in communication with each other through the three-way stopcock in the normal animal and a tendency even in these animals for blood to flow from the femoral to the carotid or brachial through the stopcock has been observed. Any such flow is however slow.

In an animal with definite arterial disease this normal difference may be exaggerated. An example of this in an old dog with intact valves is seen in the following experiment.

Experiment 1. Old fat female—weight 16 kilos. Carotid arteries obviously diseased; aorta not examined. Pulse rate 150, brachial pressure 194/164, femoral 211/170, then brachial 195/173, see figure 11. On connecting femoral and brachial together blood flowed from femoral into brachial, though slowly.

This animal duplicated closely the conditions often observed in man with arteriosclerosis, namely, a definite but not very great differential pressure. If the rise of pressure in arteriosclerosis results from increased peripheral resistance and if there was more change in the wall of the femoral artery than appeared superficially, both these factors should tend to increase the normal difference and readily explain the observed figures.

Another example of an exaggeration of the normal difference is seen in the following experiment with the production of a valvular lesion.

Experiment 2. Weight 18 kilos. Normal brachial and femoral first recorded, figure 12 *a* and *b;* pulse rate 130, brachial pressure 129/106, femoral 142/100. Aortic valve damaged with hook; pulse rate 134, brachial 190/80, femoral 217/75. Valves damaged further; pulse rate 140, brachial 159/44, femoral 212/45. (fig. 12 *c*). Mean pressures then recorded, the femoral pressure being slightly the higher and both about 70 mm. (fig. 12 *d*).

Autopsy showed one aortic cusp completely torn away, one partially torn off, one intact, and mitral also slightly damaged.

This experiment illustrates clearly how the exaggeration of the normal differential pressure with an aortic leak becomes more marked, the larger the leak. While both the diastolic and mean pressures show slight differences between the two vessels, the essential difference is the exaggeration of the systolic peak in the femoral; this high pressure is however of short duration and contributes little to the mean pressure. The upstroke

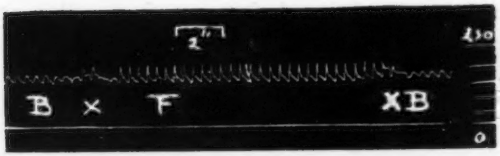

Fig. 11. Experiment 1. Brachial, femoral, brachial in old dog with sclerotic changes in vessels. (Reproduced 1/1.)

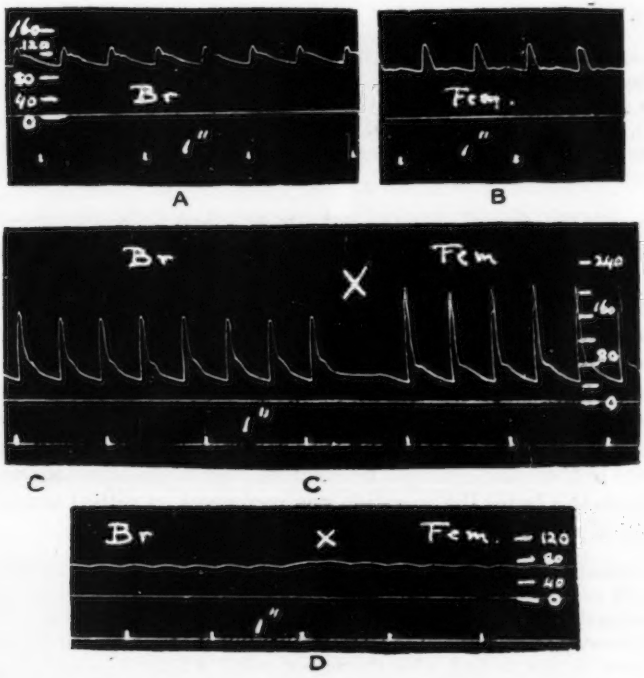

Fig. 12. Experiment 2. Dog, 18 kilo, ether. *a*, Normal brachial. *b*, Normal femoral. *c*, Brachial and femoral with regurgitation. *d*, As in *c*, but with manometer damped to give mean pressure. Time signal giving seconds. (Reproduced 2/1.)

Fig. 13. Experiment 3. Record from femoral and brachial. $ChCl_3$ anesthesia. Two valves punctured 4½ months previously. *B* indicates brachial, *F*, femoral and *X*, crossing of manometer connection through the three-way tap. (Reproduced 1/1.)

of the pulse curve is more abrupt during regurgitation then normally and a similar abruptness accompanies these lesions in man. Examples of these differences in the femoral in man may be seen in a previous paper (3) and in the radial in Feil and Gilder's analyzed curves (9). It will be noticed by comparison of figures 2 and 3 that all these characteristic changes in the nature of the pulse curve are also seen in the schema with regurgitation; so that there is a close parallel between the schema, the experimental animal and man.

An example of an experiment where the lesion was made aseptically follows.

Experiment 3. Male—Weight 15 kilos. February 6, 1923, lesions produced in two valves by a stylet.

June 20, 1923, morphine and $CHCl_3$. Brachial pressure 156/76, femoral 182/70 (fig. 13). Autopsy showed medium sized holes (about 2 to 3 mm. diameter) in two cusps and there was no arteriosclerosis in the arteries obvious to a macroscopic examination.

Although this animal, like the last, shows a lower diastolic pressure in the femoral than in the brachial, the change is again mainly in the systolic peak. That a real difference in mean pressure also existed was demonstrated by connecting the two vessels through the stopcock, when the citrate in the connecting tubes was washed out by a rapid flow of blood from femoral to brachial.

These two examples give changes so similar to those of the schema that they are most readily explained as resulting with the increased aortic velocities which must have occurred, though reflex changes in the elasticity of the femoral cannot be excluded even in the acute experiments. To exclude this factor the operation was performed on pithed animals, an example of which follows.

Experiment 4. Young active animal weight 14 kilos. Normal curves gave a pulse rate of 132, carotid pressure 210/182 and femoral 230/182 (fig. 14 *a*). A large hole was produced in the right posterior aortic cusp after which the pulse rate rose to 165, the carotid pressure to 193/125 and femoral to 232/125 (fig. 14 *b*). The animal was then pithed and the cord divided, when the pulse rate fell to 137, the carotid pressure to 115/73 and the femoral to 115/73 (fig. 14 *c*). With gradual recovery from shock and anesthesia the blood pressure rose, the record showing a pulse rate 222, carotid pressure 153/100 and femoral 170/100 (fig. 14 *d*). The right splanchnic nerve was then divided and the peripheral end prepared for stimulation. After this procedure, the pressures were both lower, and, if anything, the carotid had slightly the higher systolic pressure. At the height of a blood pressure rise resulting from splanchnic stimulation (fig. 14 *e*) the pulse rate was 270, carotid pressure 196/160, femoral 237/150. Some time later the pulse rate had slowed again to 101 and the brachial and femoral pressures were both 70/57 (fig. 14 *f*). On repeating splanchnic stimulation the differential pressure returned, and was maintained for some while after the stimulation had ceased. Figure 14 *g* gives an instance of this, the record starting 6 seconds after the end of stimulation; here the pulse rate was 189, brachial pressure

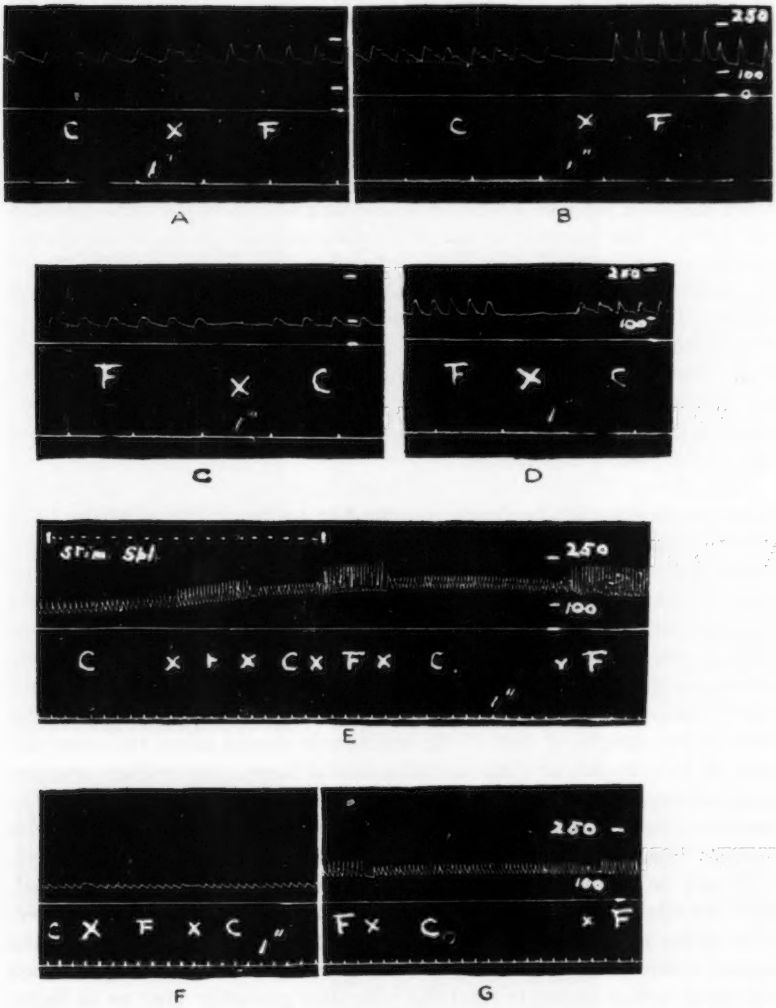

Fig. 14. Experiment 4. *a*, Carotid and femoral curves; ether. *b*, Same ether and aortic regurgitation. *c*, Same aortic regurgitation immediately after pithing. Anesthetic discontinued. *d*, Same considerably later after pithing. *e*, Same with stimulation of the peripheral end of right splanchnic nerve. *f*, Same later—feeble pulse; sometime after fall of pressure following splanchnic stimulation. *g*, Same later; period of falling pressure following another splanchnic stimulation. *C* indicates carotid. Other lettering as before. (Reproduced 1.1.)

150/109, femoral 164/95. For the last two records a cannula had been freshly inserted into the brachial, owing to trouble wih clots in the carotid.

This experiment demonstrates that a differential pressure may be recorded even after pithing when a reflex contraction in the femoral artery in inconceivable. On the other hand immediately after pithing, when the tone of the vessels was probably at its lowest, no differential pressure was found, and the difference also disappeared again later on in the experiment when the blood pressure was lowered following section of the right splanchnic nerve. The absence of the differential pressure under these circumstances is readily explicable, if the arterioles were dilated, and the transformation of kinetic energy through slowing of the stream relatively slight. The pressures recorded however even at these times were relatively high for a pithed animal, and such an hypothesis has certain difficulties. Similar high blood pressures were also obtained by Dale and Evans (6) after pithing by this method, and whatever the cause of the high pressures, it is probable that the arterioles were relatively dilated at the times that the pressures were lowest.

The effects of splanchnic stimulation also supports the hypothesis. Here the reaction was no doubt complicated, for not only must there have been increased resistance in the splanchnic vessels, but also the rate and force of the heart beat were increased, probably through adrenalin output. (Compare Sherrington (27).) A more forcible heart action should increase the velocity with which blood is thrown into the aorta and so cause a greater kinetic energy development, while the increased splanchnic resistance should result in a greater proportion of this energy being converted into pressure in the femoral region. It is unlikely that stimulation of the peripheral end of the splanchnic should affect the muscular coat of the femoral, or that adrenalin should have a particularly marked local action, so that the reappearing differential pressure is more readily explained as the indirect result of increased transformation of kinetic energy, than as the result of any local contraction of the femoral vessel.

It may be seen in this and the previous experiments that in the animal, as in the schema, the differential pressure usually varies in the same direction as the pulse pressure, but by no means always so, confirming the figures obtained by Williamson (33) on man. It is also clear that when the heart action is feeble (and the velocities probably low) as in figure 14 *f*, the differential pressure disappears.

To test whether changes in peripheral resistance have such a considerable effect on the occurrence of differential pressure, as suggested by the previous experiment, the peripheral resistance may be varied in other ways. One such method is the intravenous injection of adrenalin, which was always found to increase the differential pressure even in animals

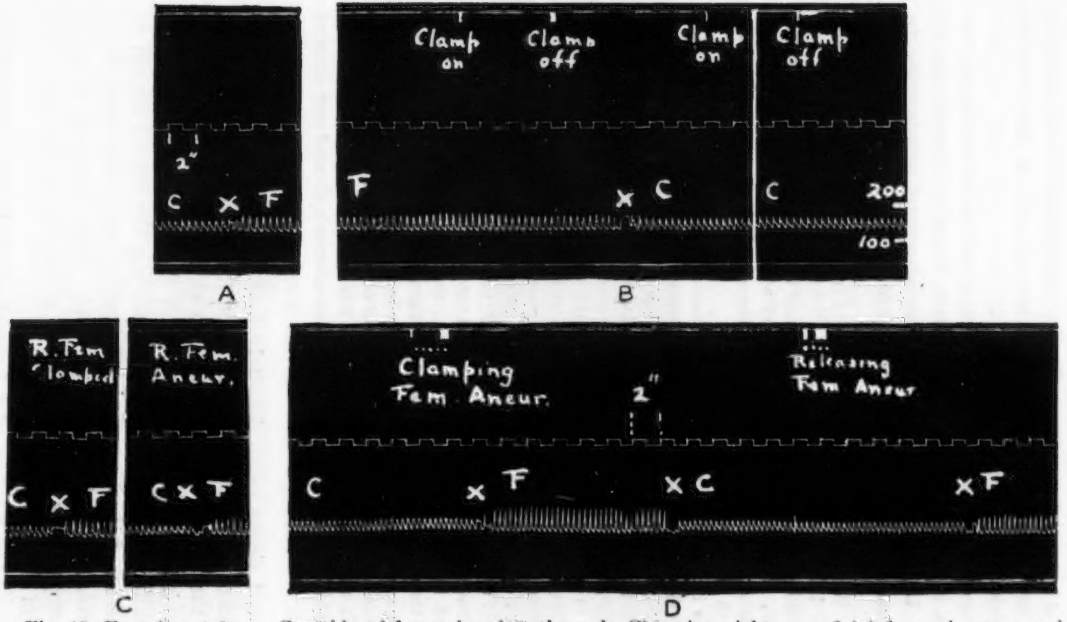

Fig. 15. Experiment 8. *a*, Carotid and femoral under ether. *b*, Clamping right superficial femoral artery and releasing while recording from femoral and repeating while recording from carotid. *c*, Record of carotid and femoral with right superficial femoral clamped, followed by record with right femoral artery to femoral vein, aneurysm open. *d*, Carotid record with femoral arteriovenous aneurysm open and also aneurysm between carotid and jugular; clamping off femoral aneurysm, and record from carotid, femoral, carotid; opening of aneurysm and record from carotid and femoral. Lettering as before but to correct signal indicating time of closure, etc., set 6 mm. to left. Note rise of pressure on closing and fall on opening aneurysm.

with intact valves. An instance of the effect of adrenalin in an animal in poor condition is given later (exper. 7); few experiments were made in this way, since again the factors introduced are complex, as they were in splanchnic stimulation.

Another method of varying the resistance consisted in the simple obstruction with a light bull-dog clamp of a large vessel to increase the resistance, or in the introduction of an arterial-venous anastomosis to lower it. An example of such an experiment follows.

Experiment 5. Weight 17 kilos. Normal record from left carotid and femoral showed pulse rate 127, carotid pressure 162/124, femoral 170/120 (fig. 15 *a*). The clamp was then applied to the right femoral artery and the femoral pressure rose from 172/126 to 188/126, returning to its previous level when the clamp was released (fig. 15 *b*). On reclamping while recording from the carotid no measurable change of pressure was seen (steady at 172/128).

An anastomosis between the right superficial femoral artery and vein was then made. After a very slight preliminary fall of pressure, the pressures returned to their previous value; before the anastomosis these were pulse rate 141, carotid pressure 175/135, femoral 194/130 (with right femoral clamped), and after, pulse rate 143, carotid 175/136, femoral 194/136. The right carotid was then clamped when with a pulse rate of 152, the pressures in the carotid and femoral were 213/164 and 220/160. A carotid-jugular anastomosis was then made in addition to the femoral, and the record showed a pulse rate of 168, carotid pressure 207/171 and femoral 233/168, after an earlier definite fall of pressure which followed the opening of the anastomosis had passed off. The two anastomoses could be closed at will. The effect of closing the right femoral artery and anastomosis is shown in figure 15 *d*. Before the clamp was applied the pressures, pulse rate, etc., were as above; a rise of blood pressure followed the application of the clamp and then partly subsided, when with a pulse rate of 161, a carotid pressure of 209/174 and a femoral of 245/170 was obtained, and on again releasing the clamp and allowing the resulting fall of pressure to recover as much as it would, the differential pressure was again reduced. At the end of the experiment, which did not last long, neither anastomosis showed any signs of blood clot.

This experiment shows quite definitely that an increase in the local resistance produced by clamping one common femoral artery in a normal animal affects the height of the systolic peak in the opposite femoral artery while having no appreciable affect on the carotid pressure, thus increasing the differential pressure, while clamping the second carotid on the contrary increased the pressure in the carotid more than that in the femoral, so decreasing the differential pressure. This is entirely in agreement with the origin of the pressure recorded at the peak of the curve from transformation of kinetic energy.

On the contrary the creation of a femoral artery to vein anastomosis gives a differential pressure which is above that seen when the femoral is conducting blood normally, but which is not above that obtained with

the femoral obstructed. Here again the results are what might be expected on the kinetic energy hypothesis, since the opening of the anastomosis should produce greater velocity changes in the vessels, but should also cause less transformation of kinetic energy into stress in the femoral region, owing to the lowering of the resistance. A carotid-jugular anastomosis should on the same theory produce greater variations in velocity particularly when added to the femoral leak and so greater development of kinetic energy, but should also diminish the amount this is transformed into stress in the carotid region, thus increasing the differential pressure; and this is exactly what it is found to do. In the presence of the carotid anastomosis which aids in keeping up high velocity changes, the femoral leak is found to be a definite disadvantage in the development of differential pressure. This effect of peripheral resistance is therefore clearly very important, and must be taken into

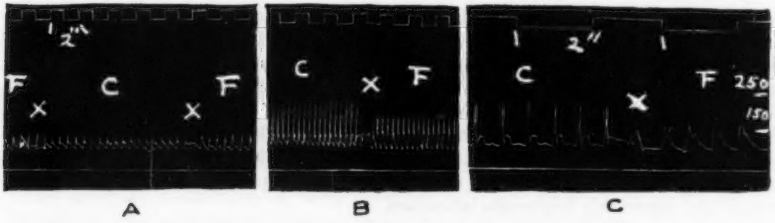

Fig. 16. Experiment 6. *a*, Femoral, carotid, femoral under ether. *b* and *c*, Carotid and femoral with ether, aortic regurgitation and right splanchnic nerve divided (Reproduced 1/1.)

consideration in the results reported both in man and animals with arteriovenous aneurysms by Lewis and Drury (22). In these they find no evidence in dogs of any increase in the circulation rate, nor any change in pulse rate, so that the average systolic velocity in the aorta remains unchanged. They do however give evidence suggesting greater velocity variations since they describe an abnormally abrupt pressure rise, similar to that seen in aortic regurgitation, and consequently the maximum velocity developed and the resultant kinetic energy is probably increased under these conditions. Since however all their detailed work was on femoral leaks, the local lowering of the peripheral resistance might be expected to off-set the effect of the increased kinetic energy. They made no differential pressure observations on their dogs, and in the patient the conditions were somewhat different, since an increased pulse rate was then seen when the leak was released, so that changes in circulation rate may have occurred. Their results on this patient also receive a ready explanation on this theory, which however does not exclude the possibility of some change on the vessel wall as an additional but not essential factor.

While the previous experiments are in entire agreement with the theory suggested, occasionally results were obtained which did not appear so readily explicable. These anomalous results were never obtained except when the experimental procedure had failed at some point so that the animal was known to be in poor condition either from hemorrhage or some change which may be classed as shock. Under these circumstances a reversed differential pressure with a carotid pressure above the femoral was occasionally seen. This reversed differential pressure differed from the more normal in that both systolic and diastolic pressures in the carotid were above those of the femoral, while in the more normal condition only the systolic pressure of the femoral would be above that of the carotid. The condition is not then an exact reversal.

The most marked case of this reversed pressure was seen in the following experiment.

Experiment 6. Excitable dog of 15.5 kilos weight. Irregular anesthesia accompanied at first by considerable hyperpnea. Artificial respiration had to be used later on several occasions before any records were taken. Normal record from carotid and femoral gave pulse rate 108, carotid pressure 148/82, femoral 160/82 (fig. 16 *a*). The right splanchnic nerve was then dissected and cut, when the difference between the two was abolished. Large holes were then produced in both posterior aortic cusps and records showed pulse rate 169, carotid pressure 242/90, femoral 205/82 (fig. 15 *b* and *c*). On attempting to pith the animal it died. Autopsy showed extensive valve lesions and apparently hyperemic intestines.

It might be supposed that such a reversed differential pressure might occur when the resistance in the splanchnic region was low, so that the aortic stream was diverted in this direction and little kinetic energy was transformed in the femoral. In this animal the pulse rate was fast and the heart beat apparently forcible, but it is difficult even so to suppose a low splanchnic resistance with such a high blood pressure. As a rule however this reversal was only seen with relatively low blood pressures, when a low peripheral resistance was probable. That peripheral resistance changes may be implicated is suggested by the fact that adrenalin injections make this reversed differential pressure disappear or change to the more common type; an example of this follows.

Experiment 7. Old female—weight 9 kilos. Considerable hemorrhage during pithing. No other operative procedure. Record showed pulse rate 135, carotid pressure 57/24, femoral 48/21. Intravenous injection of 0.025 mgm. adrenalin raised the pulse rate to 143, carotid pressure to 118/53 and femoral to 88/50, the pressures then returning to the previous level. A later injection of 0.1 mgm. of adrenalin raised the pulse rate to 173, carotid pressure to 162/83 and femoral to 164/78.

In other animals adrenalin injections have given quite large differential pressures in favor of the femoral, even when before the injection the pressures in the carotid were the higher. But even the loss of fluid through

the splanchnic system seems a difficult explanation for a reversed differential pressure observed in an animal with intact valves which had been eviscerated. Here a condition of shock seemed probable, but, though the pulse rate was extremely fast (252) the pressure was still high (carotid 144/67 and femoral 134/67). In an attempt to exclude as far as possible a leakage of the blood away through dilated vessels which might reduce the kinetic effect in the femoral vesels, in one animal evisceration and pithing was also combined with ligature of the renal vessels. A short account of this experiment follows.

Experiment 8. Weight 17 kilos. Evisceration with ligature of the renal vessels was followed by pithing. Only moderate hemorrhage. Records then showed a pulse rate of 146, and carotid and femoral pressures of 108/88 and 118/88. The artificial respiration was apparently slightly deficient for the blood pressure gradually rose till the pulse rate was 254, carotid pressure 150/130, femoral 164/134, and a little later the readings were pulse rate 264, femoral 186/158 carotid 179/164, the pressure rising steadily at the time the change from femoral to carotid was made. Ventilation was then slightly increased when the pressures fell and in a short while pulse rate was 112, femoral pressure 60/47, and carotid 57/47. Shortly after this the animal died with circulatory failure apparently resulting from disturbance of its acid-base equilibrium.

In this case, even when the heart beat was very feeble, the femoral gave a higher systolic pressure than the carotid presumably owing to the more extensive ligature of large vessels through which the blood might escape in the abdominal region, supporting to some extent the supposition that the reversed differential pressure is accompanied by low peripheral resistance.

With the evisceration and pithing, particularly with the renals tied, the animals seemed extremely susceptible to slight variations in the artificial ventilation. Perhaps this is hardly surprising in consideration of the fact that most of their means for regulating hydrogen ion concentration must have been removed by the experimental procedure. Without evisceration or aortic lesions spinal animals could be used for experiments lasting over several hours, but the circulation did not seem capable of withstanding severe aortic lesions, nor was evisceration at all well borne, whether performed before or after the pithing process. Consequently further data were not obtained. It seems probable that the reversed differential pressure is usually accompanied by low peripheral resistance, but it is difficult to explain solely on this hypothesis and some other factor, possibly Bramwell and Hill's (4) "breaker formation" may account for it.

GENERAL DISCUSSION. These experiments leave little doubt that in the schema the main factor operating is the transformation of kinetic energy into stress, and the unequal distribution through reflected waves of the pressures generated. In general these pressures will tend to be

greater the larger the mass of fluid moving toward a branch. Owing to the closely parallel changes in the schema and experimental animals it is probable that this is also an important factor in the animals, since the mass of fluid of the aorta and common iliacs directed toward the femoral should give a greater kinetic energy than would be present in vessels of the upper limbs. It has been seen that the experimental results can mostly be explained on this theory, and the evidence suggests that it is the most important factor, though probably not the only one. No adequate explanation has been brought forward for the occasional reversed differential pressure that may occur, though a suggestion of this condition is also seen in the schema with very distensible walls (fig. 9*a*).

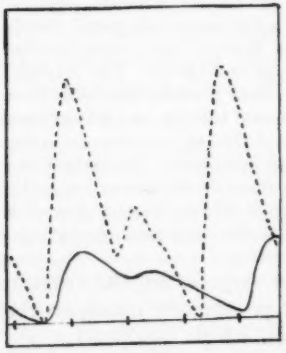

Fig. 17. Tracings of photographic records of brachial pulse from normal subject resting and immediately after exercise. Taken with glycerine tambour at elbow connected to Frank capsule. (Reproduced 2/3.) Actual time summit of pulse reached was 0.093 sec. resting and 0.078 sec. after exercise.

If the theory is correct there must be sufficient development of kinetic energy by the heart to account for the development of these pressures. Evans (8) has recalculated the kinetic energy factor in the work of the heart. He finds that in the heart-lung preparations when the output is high and the arterial resistance low, the heart may do even more work in the production of kinetic energy than in overcoming pressure, and he calculates that with exercise in man the kinetic energy may rise to 9.5 per cent of the total work. Figures obtained by Douglas and Haldane (7) on Douglas after exercise give similar values. Thus with a pulse rate of 137 and an output per beat of 136 cc. the duration of the expulsion period should have been according to Lombard and Cope (24) 0.187 second. Calculation using the same figures as Evans for mean blood pressure and diameter of the aorta, gives a mean systolic velocity of 1.5 meters a second and a kinetic energy factor of 6.5 per cent of the total work of the left ventricle. Actually the velocity curve during the expulsion period is complicated and probably resembles in character those obtained in large vessels by various observers (see Hürthle (17)). That the systolic ejection is by no means at a uniform rate is also shown by the ventricular volume curves obtained by Wiggers and Katz (32). The maximum systolic velocity probably was considerably above 1.5 meters per second and the kinetic energy therefore much above the 6.5 per cent of the total work calculated above.

With the development of rapid velocities a more abrupt rise in the pulse curve similar to that described by Feil and Gilder (9) in aortic regurgitation and by Lewis and Drury (22) in arteriovenous aneurysms might be expected. Such an abrupt rise, comparable to that seen in these conditions, is readily observed after exercise. Figure 17 reproduces tracings of photographic records obtained from the brachial in a normal subject immediately before and after exercise; the similarity of the normal curve to those of the above pathological conditions is obvious.

Assuming therefore that the velocities in man at their maximum are sufficient to account for the development of considerable kinetic energy, the differential pressures observed in clinical cases may be readily explained, but the normal identity of brachial and femoral pressures in man, as described by L. Hill and his co-workers, becomes somewhat difficult to correlate either with the schema or with animal experiments. To meet this objection an optical method for simultaneous record of brachial and femoral pressures in man has been developed, and figures so obtained show that even in the normal individual lying down at rest, the femoral systolic pressure is in reality higher than that of the brachial, and that this normal difference is very much exaggerated by exercise. Figures obtained by students working under my direction by this method will be published shortly. There is therefore no discrepancy between the experimental observations and those to be anticipated from the theory.

Whenever there is kinetic energy transformation of any magnitude producing a sudden development of pressure the pulse curve must become peak-like in character. Such a type of curve is seen in all these conditions, and it can probably be stated that, in the absence of a pulse curve of this type, kinetic energy transformation is relatively slight in the neighborhood of the particular vessel examined.

Measurements of the blood vessels in man show that the changes in velocity in the large vessels are relatively slight. Poirier (25) finds that the aorta is even of somewhat greater cross-sectional area than the sum of its branches, but measurements given in other text books together with those made by Ballance and Edmunds (1) show that there is comparatively little change in cross sectional area between the ascending aorta and the sum of the descending aorta, innominate and left carotid or between the abdominal aorta and the sum of the common iliacs. The mean velocity must therefore remain approximately constant throughout the larger vessels. On the other hand there is a considerable slowing of the stream with the broadening of the system in the peripheral branches; thus measurements of the small artery reproduced from Spalteholz by Krogh (20) show an increase in cross sectional area of about 200 per cent within the primary branches of this vessel. The physical factors concerned in such a broadening of the stream with transformation of kinetic

energy are clearly stated by Starling (28). Such factors as he has considered should however still be active when the arterioles and capillaries are dilated, but the experimental evidence suggests that the differential pressure is much more marked, when the arterioles are constricted and the resistance high. Consequently resistance to the flow created by constricted arterioles is probably more important than the mere change in energy distribution brought about by the broadening of the stream.

The conclusion that reflected waves play a very impotrant part in the phenomena of the circulation has also been reached by Hürthle (19), in searching for an explanation of the want of parallelism in animals between the velocity curve and pressure curve when the peripheral resistance is high. He finds under these circumstances that the velocity in the periphery during the systolic phase is much higher than would be anticipated from the aortic pressure, a result in entire agreement with the kinetic energy theory here advanced.

The original observations of L. Hill on the effect of local heat in abolishing differential pressure are also readily explained by this theory, since with the dilatation of the vessels and supposed change in elasticity, there must also have occurred an arteriole dilatation, lowering the peripheral resistance and diminishing transformation of kinetic energy. Hill's theory cannot however be discarded completely. Hürthle (18) has shown that the femoral artery is normally less distensible than the carotid, and Leschke (21) has described a special thickening of the femoral vessels as compared with others in cases of aortic regurgitation of long standing. Leschke thought this thickening of particular importance since he was unable to demonstrate a differential pressure following acute aortic lesions in dogs. With the latter conclusion my results are in absolute disagreement. Lewis and Drury (22) have also concluded that the big differential pressure they observed in a case of arteriovenous aneurysm was due to vascular changes, since it was still considerable, when the aneurysm was compressed. While their results can be best explained on the kinetic theory and must have been modified by the sudden production of a considerable change in peripheral resistance by the obstruction of the aneurysm, still their figures do suggest a presence of some such arteriosclerotic change in the vessel. On the other hand with general arteriosclerosis in man where irregular hardening of vessels must often occur, any considerable differential pressure is not common. It would therefore seem more probable that the increased sclerotic changes that are found in the femoral under these conditions are produced by the additional stress to which the vessel is exposed, and are the result rather than the cause of the differential pressure. There can be no doubt, however, that if these arteriosclerotic changes occur locally they must considerably exaggerate the pressure differences.

If a reflex change in the vessel wall, as Hill suggested, were the essential cause in the production of the phenomenon, then an increased pulse wave velocity should be found in these vessels. Any figures in the literature obtained on such cases in young individuals without arterial disease show no evidence of this, and further figures obtained by others in my laboratory and as yet unpublished confirm the absence of any such increase in pulse wave velocity in the femoral vessels.

The "breaker formation" theory of A. V. Hill and Bramwell has been mentioned; it is probable that it plays a part in causing or accentuating the phenomenon, but again does not appear essential. It perhaps is the most likely cause of the anomalous conditions, which have been mentioned but not explained here.

One must conclude therefore that the differential pressure in aortic regurgitation is mainly a water-hammer action resulting from the changes in the circulation described by Corrigan (5) in his classical account of aortic regurgitation (where he explains the great pulsation of the vessels as due to the blood being sent along as "a rushing current" owing to the relative emptiness of the large vessels), and is merely modified by variations in the character of the arterial wall and peripheral resistance and possibly by "breaker formation." Even in the normal subject the effect is slightly in evidence, and an exaggeration of this may be produced by many other conditions, including variations in blood velocity and local resistance produced by arteriovenous anastomosis, exercise and probably other physiological or pathological conditions, including hyperthyroidism. The condition is therefore well named—water-hammer pulse.

Finally I should like to express my thanks to many of my colleagues for the assistance they have given me, and especially to Dr. E. Lodholz and Dr. M. Jacobs for pointing out to me some of the mechanical principles concerned; to Dr. A. E. Livingston for giving me the benefit of his experience and performing some eviscerations for me, to Drs. J. E. Sweet and G. W. Norris for stimulating my interest and presenting to me the clinical problem, as well as to Sir Thomas Lewis and Dr. J. C. Bramwell for keeping me informed of their work even before publication.

CONCLUSIONS

1. A schema consisting of rubber tubing with branches will give a slightly higher systolic pressure in the "femoral" vessel than in the "carotid" under intermittent distention and this difference is enormously exaggerated if the conditions of aortic insufficiency are simulated.

2. These differences in the schema appear to be dependent on the kinetic energy of fluid in rapid motion, in fact to a "water hammer" action.

3. In dogs the normal differences between the femoral and carotid or brachial may be exaggerated experimentally by the production of an aortic lesion, by making an arteriovenous anastomosis in the neck, and also by such means as adrenalin injection or stimulation of the peripheral end of the splanchnic nerve, but this normal difference in pressure is not necessarily increased by the production of an arteriovenous anastomosis in the leg.

4. The obstruction of the blood flow in a large vessel produces a rise of systolic pressure which is much greater in the vessels relatively close than in those more remote, produces in fact a differential effect.

5. A differential pressure with aortic regurgitation has been observed in dogs immediately after the production of the lesions and sometimes even when the animal has been pithed, so that vascular reflexes are excluded.

In animals with regurgitation in which the peripheral resistance appears to be low and the heart action forcible, a reversal of the differential pressure may be sometimes seen. It is suggested that this may accompany an increased distensibility of the system or a low peripheral resistance in the abdominal region. A similar but much slighter reversal in the schema is obtainable.

7. Most of the types of differential pressure described are explicable as the result of kinetic energy converted into stress when the flow meets with resistance, the differences between different vessels depending on the relative degrees of slowing and relative masses of the blood involved. Other types may be due to changes in the vessel wall, but such changes again probably act by the effect produced on the transformation of kinetic energy into stress.

8. It is shown that an origin of a high femoral pressure from kinetic energy transformation, the effect disappearing when the slowing is diminished by lowering the peripheral resistance, would explain the results obtained by Hill with warm baths just as well as his own theory of elasticity changes, and that both factors probably assist in the disappearance of the differential pressure.

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Fig. 18. Reproductions of *C*. Tigerstedt's curves from a rabbit; *a* carotid, *b* femoral. For comparison with figures 2 and 20.

Fig. 19. *a*, Photographic record of records from schema arranged as for figure 2, but with slower pulse rate. Dotted lines show approximate position of the zero line and of the pressure (108 mm. Hg) available from the bag (reading to the bottom of curves). Figures over the highest points of the curves indicate approximate maximum values reached. Dicrotic notch marked *3*. (Reproduced about 2/3.) *b*, Record as in *a* but with reservoir raised to deliver at 140 mm. Hg.

Fig. 20. *a*, Record as in 19 *b* but with pulse rate increased raising diastolic pressure and reducing pulse pressure. *b*, Record as in *a* but with regurgitation sufficient to reduce diastolic pressure to zero. (Reproduced about 2/3.)

Fig. 21. Photographic record from central end *C*, soft rubber "femoral" F_1, and glass "femoral" F_2 of system shown in figure 10.. Scale on record indicates approximate zero and available pressure from bag (112 mm. Hg). Figures indicate approximate maximum pressures recorded. Dicrotic notch marked *3*. Time marker indicates 1/10 sec. (Reproduced 1/1.)

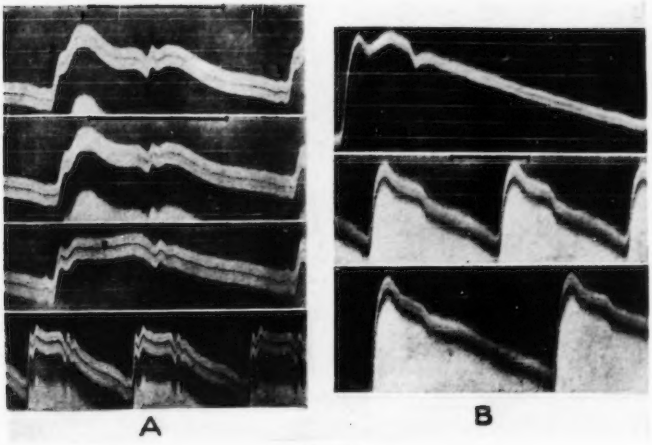

Fig. 18

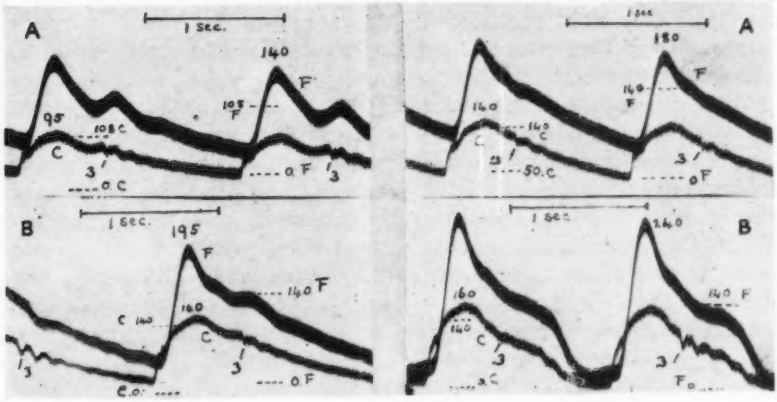

Fig. 19

Fig. 20

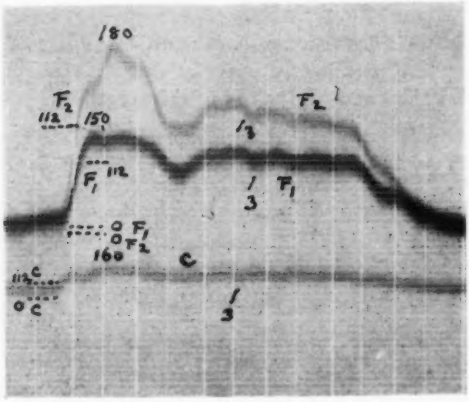

Fig. 21

THE RESPIRATORY WAVE IN ARTERIAL BLOOD PRESSURE

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There are, in all probability, few physiological problems of such apparent simplicity which have resisted solution as long as has the problem of the causation of the respiratory wave in arterial blood pressure. To the casual thinker it seems strange that one cannot readily correlate the variations in arterial pressure with the phases of respiration in which they occur and thus come to a satisfactory solution of the problem. An examination of the results of various workers in establishing this relation, however, shows conclusively the inadequacy of that means of explanation.

Lewis (1) has made a tabulation of the data offered on this point by nineteen investigators of whom four describe an inspiratory rise, nine an inspiratory fall, and six have obtained variable results which they attribute to the type of breathing, that is, thoracic or abdominal, and to the lengths of the phases of respiration in their various experiments. There is a moderate amount of agreement between authors with regard to an inspiratory rise during deep abdominal breathing, and an inspiratory fall during thoracic breathing (1), (2). Lewis has shown, moreover, that if the phases of thoracic breathing are prolonged, the rise in arterial pressure, which would otherwise have come on during expiration, now comes on during inspiration, thus illustrating a point which he neglected to emphasize, viz., that the rises and falls in blood pressure may not necessarily be dependent upon simultaneous respiratory movements; in fact, as we will show below, they frequently are not.

The mere fact that abdominal and thoracic types of breathing may produce diametrically opposite effects, proves that the variations in blood pressure are dependent upon more than a single factor. In attempting to solve the problem of the cause of the respiratory wave it becomes necessary to consider the various factors which might be responsible for the phenomenon. This will be done in the next section, and in the third section the various factors will be correlated.

ANALYSIS OF FACTORS CONCERNED WITH RESPIRATORY VARIATIONS IN ARTERIAL PRESSURE: A change in systemic arterial blood pressure can conceivably be due to two things; first, a change in the peripheral re-

sistance, and second, a variation in the output or force of the pump of the system, the left heart. These factors as modified by respiration will be considered in detail.

Constricting the bore of the vessels through which the arterial blood passes would cause a rise in the pressure of the system. If this constriction were rhythmic and intermittent it might produce a variation similar to the respiratory wave in arterial blood pressure. Several factors dependent upon respiration might produce such a constriction. The contraction of the respiratory muscles might constrict the capillary bed within them temporarily, but the respiratory musculature accounts for so small a fraction of the total capillary bed of the circulatory system that such an effect would be negligible, and Krogh has shown that the capillary bed increases in active muscles.

Next, it has been argued that the contraction of the diaphragm might constrict the aorta enough to raise the arterial blood pressure. If this were true the pressure in the arteries below the diaphragm should fall when that in the arteries coming from the aorta above the diaphragm would rise. In other words, the respiratory wave as recorded from the carotid would be the reverse of that recorded from the femoral artery. It has long been known that this is not the case.

It has also been suggested that the increase in intra-abdominal pressure coincident with the descent of the diaphragm in inspiration might obstruct the flow of blood. It has been shown conclusively, however, by de Jager (3) that variations in abdominal pressure of the order met with in normal respiration exert no effect on arterial blood pressure. If this condition were true, likewise the respiratory wave in the carotid should be the reverse of the femoral.

In considering the peripheral resistance to the flow of blood it is necessary to consider the fact that the relative pressure against which the heart must work can be altered without changing the condition of the peripheral vessels at all. By lowering the pressure in the thorax and thus in the heart itself, the difference of pressure between the heart and the peripheral arteries is increased. Therefore, the heart must do more work to maintain the arterial pressure at its previous level. This fact was emphasized by Ludwig (34) and it might seem at first thought that it could account for the whole of the respiratory wave in blood pressure. However, on analysis one sees that if it were the important factor one should find a fall in blood pressure during inspiration since the intrathoracic pressure is lowered in inspiration and therefore the "relative pressure" is increased. But when one produces inspiration by lowering the intrathoracic pressure one finds the major effect on the blood pressure is a rise (see fig. 6). One does, however, obtain a short preliminary fall which may possibly be partly explained by this increase in the relative pressure against which the heart must work.

Why is it that in spite of the lowered intrathoracic pressure and consequent increased "relative pressure," the blood pressure does not fall? The lowered intrathoracic pressure increases the blood inflow to the heart by facilitating the venous return, and this, as Knowlton and Starling (4) have shown, causes the heart muscle to respond with greater work, and therefore, it would tend to maintain its previous output of blood even if the work it must do to accomplish that end is increased. This factor will be considered later in more detail.

There is another way in which the peripheral resistance might conceivably be altered, that is, by changes in the tonus of the vessels due to changes in the vasomotor system. Few or no physiologists have regarded this seriously as a factor in producing the respiratory wave, although there are rhythmic variations in tone producing "les variations de troisieme ordre" which Mougeot and Petit (5) attribute to a vasoconstriction to compensate for a gradually falling venous pressure. The Traube Hering (6) waves are of this type also (7). These waves extend over several respiratory waves and are clearly of an entirely different nature.

Since the effect of increasing the resistance in the capillary bed of the respiratory muscles is negligible; since the effect of changes in intra-abdominal pressure of the magnitude observed in normal respiration would have no direct appreciable effect; since the diaphragm does not constrict the aorta enough to cause a rise in arterial pressure, and since the possible effect of the increased relative resistance caused by lowered intra-thoracic pressure is not observed to any appreciable extent experimentally, one may conclude quite safely that changes in peripheral resistance are not of great importance in explaining the causation of the respiratory wave in blood pressure.

There remains, then, simply the factor of changes in cardiac output and efficiency to explain the problem. Several factors may alter the output of the left heart. Starling (4) has shown that the output of the denervated heart is determined by the blood inflow and by the pressure at which the venous blood is delivered to the heart. Thus, the first general factor to be considered is the inflow into the left atrium. This factor is influenced first by the state of the vessels in the pulmonary system, and second, by the output, and thus primarily the supply, to the right half of the heart.

After Starling's fundamental work on the general factors governing cardiac function had been done, Henderson and Barringer (8) investigated the actual application of the more general laws to normal animals with the thorax closed. They found that the difference between the pressure in the thoracic cavity and that in the atria and veins was the significant factor in determining the filling and consequently the output of the heart. This value they called the "effective pressure." They found that changes in "effective pressures" below a certain maximum found experimentally

to be 50 mm. of H_2O, had a much more profound effect on the cardiac output than did changes above that value. This pressure they called the "critical pressure," and they maintained that changes above this level were unable to affect the cardiac output. It has since been shown, however, by Wiggers (9) that these changes do affect the heart output, but to a lesser extent than do changes below the "critical" level.

These observations were made upon systemic venous pressure but it is obvious that the same general principles hold for the pulmonary venous pressure in relation to the output of the left heart. But the understanding of the inflow to the left heart is complicated by the fact that antagonistic forces are at work for its supply depends not only upon the output of the right heart, but also upon the condition of the vessels in the lungs. When the resistance to the flow of blood through the lung vessels is increased, the supply to the left heart will be reduced. When the resistance is decreased the supply will be increased. However, it so happens, as will be shown later, that the same factors which increase the output of the right heart increase the resistance to the flow of blood through the lung vessels and therefore the effects are partly neutralized. The stronger factor only shows up in the end result.

It is necessary to analyze the problem completely in order to have an adequate understanding of it in spite of the fact that some factors are more important than others. Therefore, one must consider the question of the effect of the various phases of respiration upon the condition of the blood vessels in the lungs.

It is very obvious that the alternate stretching and relaxing of the lung wall accompanying inspiration and expiration must have an effect upon the capillaries embedded in that wall. Just what that effect is has been the subject of many a lengthy dissertation, and is still a matter of dispute.

Very early, Haller (10) expressed the belief that the capillaries in the lung wall were expanded by the stretching of the lung in inspiration and that consequently the circulation was accelerated during inspiration. Very shortly, however, Poisseuille (11) actually measured the flow of blood from the pulmonary veins when the lungs were artificially perfused and he found that the flow was greatly diminished when the lungs were inflated. His work was immediately criticised on the score that he was not simulating physiological conditions; that in normal inspiration the lungs were expanded by a decreased pressure on their exterior, and that under that condition the outer layer of the lung would be pulled from the inner and the capillaries dilated. Quincke and Pfeiffer (12) put forward this objection and supported it by presenting some experiments in which they placed a lung in a closed vessel so arranged that they could inflate the lungs by lowering the pressure in the vessel. They connected the

pulmonary artery and veins to two glass tubes extending out of the vessel and filled the whole system with a perfusion fluid. They then determined the capacity of the lung vessels in inspiration and expiration by noting the fall in the level of the fluid in the tubes. They found, to be sure, that capacity was very slightly greater in the inspiratory condition than in the expiratory, but their results are insignificant because their experiment was just as unphysiological as the experiments they were criticising, for their experiments corresponded to opening up the thorax of an animal, putting the lungs only in a vessel whose pressure they could vary, thus producing inspiration and expiration. Under physiological conditions not only the lungs but also the head of pressure in the vessels on both sides, i.e., the pulmonary artery and veins, as well as the heart, are exposed to the same lowered pressure as the lung. This was pointed out by Funke and Latschenberger (13) who showed that if the pulmonary vessels were exposed to the same lowered pressure as the exterior of the lungs the blood capacity decreased in the inspiratory condition. Bowditch and Garland (14) repeated these experiments and corroborated Funke and Latschenberger's findings, making the single exception that when the external pressure was lowered very slightly,—one or two millimeters of mercury,—there was sometimes a slight increase in the capacity of the lung vessels, otherwise inspiration always produced a decrease in the capacity of the vessels. De Jager (15) obtained results in harmony with these.

Tigerstedt (16), Heger and Spehl (17) and many others still contended that the lung capillaries were expanded in inspiration. Heger and Spehl based their belief upon their observations on the proportion of the total blood volume present in the lungs of animals killed in inspiration and in expiration. They opened the thorax of rabbits under artificial respiration, passed ligatures around the venae cavae and the aorta, then closed the thorax and allowed the animals to breathe normally. They then tightened the ligatures in the inspiratory phase in some and the expiratory phase in others and determined by measurements of the hemoglobin the proportion of the total blood of the body found in the lung vessels in each case. They found a greater proportion of the blood in the lungs when the ligatures were tightened in inspiration than when fixed in expiration. They interpreted this to mean that the lung capillaries were expanded in inspiration. However, that is not the most plausible explanation. It may mean, and it seems more likely that it does, that the atria and great veins which they included in their ligatures, held more blood in inspiration than in expiration, and that when the thorax was opened and the pressure equalized, the lung capillaries were expanded by this excess of blood in the larger vessels. The differences were only about 2 cc. for a large rabbit which might easily be accounted for on this basis. Until this factor

is eliminated one is quite justified in ruling out their results as an argument in favor of an increased blood flow through the lungs during inspiration.

Microscopic sections of lungs fixed in an inspiratory and expiratory condition have been found by Cloetta (19) to show that the bore of the vessels is much decreased in natural inspiration. This decrease is so marked that there can be no mistake about it, yet it has been almost totally disregarded in recent literature. A decrease in the diameter of the vessels means a very great increase in the resistance offered to the flow of blood, especially since there is a concomitant lengthening of the vessels.

Stewart (18) has applied his method of velocity measurement to the determination of the rate of flow in the lesser circulation. His work was done largely on animals with the thorax open and under artificial respiration where he found inflation of the lungs to decrease the circulation time. Under conditions of normal respiration he found the circulation times in inspiration and in expiration to be nearly the same. It is impossible to make any definite statement about what that finding means because, as will be shown later, the phases of inspiration and expiration do not show pure effects over the whole of their periods and the results one would obtain by Stewart's method would depend entirely upon what part of inspiration or expiration or parts of both were included in the time. Therefore, his results cannot be directly transferred to this problem.

More recently, Wiggers (21) and Schafer (22) have attacked the problem of the pulmonary arterial pressure. Wiggers, in particular, has found that in the beginning of inspiration there is a fall in pulmonary arterial pressure. He interprets this to mean that there is a decreased resistance to the flow of blood through the lungs in inspiration. However, his published tracings show very consistently that there is a fall for only one or at most two heart beats at the beginning of inspiration and it is followed by a rise. This rise probably constitutes the real inspiratory effect as data to be presented in this paper will indicate. The preliminary fall probably represents a carried over effect of expiration and the real inspiratory effect requires several heart beats to establish itself.

It becomes of interest to see why investigators have come to such contrary opinions on this question. Most, if not all, of the actual observations that have been made, have been proven to be correct. It has only been in the interpretation of the facts that errors have been made. Many of the interpretations were based upon the preconceived notion that inflation of lungs by decreasing their external pressure should stretch the lung tissues apart so as to dilate the capillaries, while artificial respiration should compress them. This seems to have been the prevalent opinion among workers on the pulmonary circulation. It is, however, erroneous, as can readily be shown.

When the lungs are inflated by decreasing the pressure outside them, they are in reality being inflated, as everyone recognizes, by a greater air tension within them than exists in the pleural space. For example, if the atmospheric pressure, and therefore the intrapulmonic pressure, is 760 mm. of Hg, and the intrathoracic pressure is lowered from that value to 740 mm., the lung is going to be expanded a definite amount because of the increased tension inside, the exact expansion being dependent upon the elastic tension of the lung as well as the pressure. Now, the pressure that is effective in producing inflation is in reality only the difference between the intrapulmonic and intrathoracic pressures. Since it is only the pressure difference that is important it should be immaterial whether the

TABLE 1

Showing the tension in millimeters of water to which the lung tissue is exposed when expanded to equal extent by artificial and natural types of respiration. All the figures are averages of three readings for each experiment. Four different lungs were used in these experiments.

	EXPANSION PRODUCED BY INCREASING INTRAPULMONIC PRESSURE			EXPANSION PRODUCED BY LOWERING INTRATHORACIC PRESSURE
Experiment	Intrapulmonic pressure (positive)	Intrathoracic pressure (positive)	Difference=tension on lung tissue	Intrapulmonic = 0 therefore figures = tension on lung tissue. Intrathoracic (negative)
1	95	48	47	46.5
2	100	29	71	72.0
3	180	92	88	84.0
4	119	27	92	94.0
5	178	74	104	100.0
6	160	53	107	107.0
7	158	46	112	112.0

pressure differences were between 740 and 760, as in normal inspiration or between 760 and 780, as in artificial respiration. The tension on the lung wall is the same in each case. There is no reason why the effect on the capillaries in the lung wall should be different. As we have seen, Poisseuille (11) with the pressure differences corresponding to the 760 to 780, found exactly the same decrease in capacity of the lung vessel that Funke and Latschenberger (13) found under corresponding conditions with pressure differences from 740 to 760 mm. Hg.

To our knowledge, the very evident theoretical consideration that the expansion of the lung and lung capillaries must be the same for a given pressure difference no matter if the range were from 740 to 760 or from 760 to 780, has never been put to an objective test. In order to do this, we tried to get measurements of intrathoracic and intrapulmonic pressures at equal expansions of the lung produced by natural and artificial respiration. On the animal it is rather difficult to be sure of equal expansion

but it is comparatively easy on a model. This model was a wide mouthed bottle whose bottom was cut off and closed with rubber dam. The tube passing through the cork to which the trachea was tied, had a side outlet to a water manometer, and another tube through the cork was connected to a second manometer. The difference in pressures between the manometers would indicate the elastic stretch of the lungs and thus the tension to which the blood vessels were submitted. In these measurements, atmospheric pressure was taken as zero and as table 1 shows, the difference between the pressures is the same for equal expansion of the lung whether the expansion is produced by artificial or natural respiration. Ranges up to 112 mm. water on each side of zero have been used.

Therefore, we may consider it quite well established that, barring any effect upon the return of systemic blood to the thorax and consequent change in cardiac output, inspiration tends to diminish the flow of blood through the lung vessels. Expiration would tend to increase the flow by decreasing the resistance.

These relations hold true only so long as the supply to the heart remains constant. This, however, is not the state of affairs in natural breathing. Actually the supply to the right heart is changing at the same time that the condition of the lung vessels is changing so there are two independent, in fact, antagonistic variables to be considered. The increased cardiac output actually entirely counteracts the effect of increased resistance in the lung capillaries upon the volume flow through the lungs. This factor will now be considered in more detail.

Another most important factor in inspiration to be taken into account is the effect of a changing pressure in the thoracic cavity upon the venous return to the right heart. It has long been recognized that the respiratory movements have an effect upon the flow of systemic venous blood. Haller in 1760 (10) observed the swelling of the great veins of the neck in expiration and their collapse during inspiration. De Jager in 1884 (3) recognized a suction pump action by the thorax in inspiration. More recently, Burton-Opitz (23) and Hooker (24) have observed the same thing and actually measured the changes of volume flow and found that the lowered intrathoracic pressure in inspiration markedly increased the flow of blood into the thorax. The increase in intra-abdominal pressure simultaneous with the inspiratory fall in intrathoracic pressure causes a forced flow of blood from the abdomen and so tends to increase the effect of the lowered intrathoracic pressure (23), (3). Lewis (1), however, found that normal inspiration did not raise the intra-abdominal pressure sufficiently to cause any significant change in the venous outflow and that only forced inspiration could bring about such an effect.

The effect of an augmented venous supply to the right atrium is easy to predict. It is undoubted that lowered intrathoracic pressure will

increase the venous return to the heart. Henderson and Barringer (8) and Wiggers (9) showed that the filling of the right atrium was dependent upon two factors, the venous pressure and the intrathoracic pressure, whose difference they termed the "effective pressure." The venous pressure never goes below atmospheric pressure because the pressure of the atmosphere is always playing upon the venous channels of the most of the body. The intrathoracic pressure varies over a much wider range than do the venous and auricular pressure, however, and therefore it is the most important factor in determining the "effective pressure" and thus the filling of the heart. The effective pressures show a range, according to Wiggers, of 43.6 mm. H_2O in expiration to 63.1 in inspiration. It is evident that in the range between 43 and 63 there would be ample opportunity for large variations in cardiac filling and output. It is impossible to assign to their proper places the various factors concerned in the increased venous supply in inspiration until more experimental data are obtained. It can only be said that Luciani (25), de Jager (3) and others, believe that increased abdominal pressure plays a large part in forcing blood into the thorax, whereas others, Eppinger and Hofbauer (26), believe that diaphragm contraction constricts the quadrilateral foramen through which the inferior vena cava passes, enough to counterbalance the effect of increased pressure. On the other hand, Burton-Opitz (23) believes that the flow from the head region and the abdominal cavity itself is increased because of the suction pump action of the lowered intrathoracic pressure in inspiration, whereas the flow from the lower extremities is impeded by the coincident increased abdominal pressure. Without complete simultaneous measurements of the flow of blood through the inferior and superior venae cavae and azygos veins under various conditions of pressure in the thorax and abdomen, one cannot make definite statements regarding the relative importance of these various factors.

In experimental work till now it has been assumed that the increased flow of blood into the thorax in inspiration was due to the fact that the pressure was lowered in that condition and that fluids seek the place of lowest tension, consequently the blood flowed more rapidly into the thorax. That is an altogether reasonable assumption, but it is not free from criticism until it is proven experimentally that the effect is not wholly or in large part the result of diaphragm action on the blood reservoirs in the abdomen, in particular, the liver and portal circulation. It is conceivable and has been argued that the diaphragm movement does play an important rôle in this process. To investigate this question the intrathoracic pressure was varied independently of diaphragm movement and the effect upon the systemic arterial pressure observed. To accomplish this, dogs anesthetized with ether were given curari to paralyze the respiratory muscles. Artificial respiration then had to be maintained. Two glass tubes of a

cannula type were inserted through the chest wall into the pleural cavity and fastened in place by sutures in the skin. One of the tubes was connected with a manometer whose movements were recorded on a smoked drum, and the other was connected through a trap with a source of suction so enabling one to vary the intrathoracic pressure at will and maintain it for any length of time at any level.

Reference to figure 6 will show that lowered intrathoracic pressure after causing a slight fall, brings about a great increase in arterial pressure, showing a large increase in cardiac output, thus indicating a similar increase in the venous return to the left atrium due to an increased supply to the right heart. In fact, there can be no doubt of the increased venous return because cardiac output is affected by only two factors; first, its supply, and second, the rate and force of its beat. Measurement of the curves shows no significant increase in rate. Unless the force of each contraction were markedly altered by nervous factors one would conclude that the venous supply had been augmented.

To demonstrate the existence or non-existence of nervous factors, these procedures were repeated on animals whose vagi and nervi accelerants had been cut. The vagi were cut in the neck and the effect of the nervi acclerants eliminated by removing the stellate ganglia by approaching them from the dorsum of the animal, a method described in detail by Sherrington (27). Under these conditions the same increase in blood pressure was obtained when the intrathoracic pressure was lowered, constituting a demonstration of the fact that the rise was entirely due to increased venous supply during the period of decreased intrathoracic pressure.

Lewis (28) has attributed most of the respiratory variations in blood pressure to changes in the intrapericardial pressure. It was found on repetition of his work that his findings were correct regarding a rise in blood pressure when the intrapericardial pressure was lowered and a fall when it was raised. He has published records showing that in natural respiration with the chest closed there are variations in the intrapericardial pressure. He concludes that since there are these variations in intrapericardial pressure, they must be responsible for the respiratory waves in arterial pressure. His point is well taken insofar as it draws attention to the fact that the pressure on the chambers of the heart, the atria in particular, is a large factor in determining the venous supply to the heart, and hence its output. But it is badly taken insofar as it emphasized the pericardial chamber as the important factor in the process.

The pericardial cavity is merely a chamber within a chamber and is utterly incapable of changing the pressure within itself. Its pressure is entirely determined by the intrathoracic pressure, so it is unnecessary to speak of intrapericardial pressure as something apart.

It must be stated that there are undoubtedly other factors involved in the increased flow of blood to the heart in inspiration besides that of lowered intrathoracic pressure. Kuhn (29) showed that the respiratory waves in arterial pressure persisted after opening the chests of animals, and de Jager (3) showed that this effect was largely the result of compression of the abdominal venous channels. The fact of a wave in arterial pressure coincident with the respiratory movements of animals with opened thorax, and the further observation of Fredericq's (30) that the wave did not entirely disappear when the abdomen was opened, were verified. Fredericq suggested that this might be a result of cardiac acceleration or release of inhibition. In order to test this, the heart was denervated. Similar variations were found, thus eliminating the nervous factor in this process (fig. 1). It is impossible to say definitely just what is responsible for this variation but it is evident that only two factors could be causative ones. Either the venous inflow into either auricle is altered, or the arterial resistance in the systemic circuit is changed by the muscular movements. Of these two, the former is by far the more probable cause. It is possible that the movement of the thorax compresses the azygos or other veins and urges the blood toward the heart momentarily.

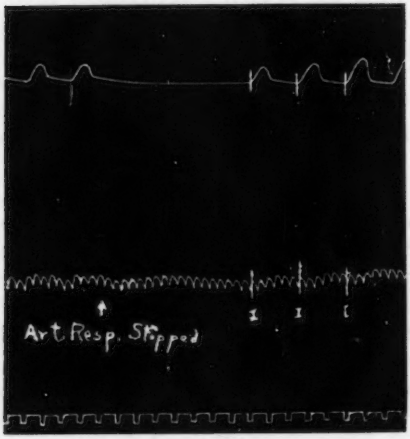

Fig. 1. Effect of cessation of artificial respiration on respiratory wave of blood pressure. Dog, weight 12 kilos. Denervated heart. Thorax and abdomen open. Upper curve, respiration recorded by balloon between diaphragm and liver connected to Marey tambour. Upstroke—inspiration. Lower curve—carotid blood pressure. Inspiratory movement begins at *I*. Bottom line—time in seconds. Shows respiratory wave when thorax and abdomen are open.

The fact that part of the respiratory wave present with the thorax open persists after opening the abdomen does not mean that normally the action of the diaphragm on the abdominal contents plays no part in the process. To test the possible means of affecting the blood flow, the effect of gentle pressure on the liver upon the arterial pressure was observed (fig. 2). It was found that rhythmic pressure upon the liver always produced a rhythmical rise and fall in pressure, thus demonstrating the extreme susceptibility of the hepatic blood channels to changes in pressure.

It is evident that many factors may influence the venous return to the thorax, and it is impossible, until more data are accumulated, to assign quantitative values to each factor. It appears safe, however, to say that the intrathoracic pressure plays the largest part in determining the rate of flow.

There is one other factor which might have a slight effect on the filling of the ventricles, and that is, the effect of the change of intrathoracic pressure on the capacity of the great veins and atria. Alterations in pressure such as normally occur in the thoracic activity, must have a much greater influence on the thinner walled vessels and atria than on the thicker walled vessels and ventricles. These changes will all be part of the latent period in the filling of the ventricles and will play a part in the latency of the changes described below.

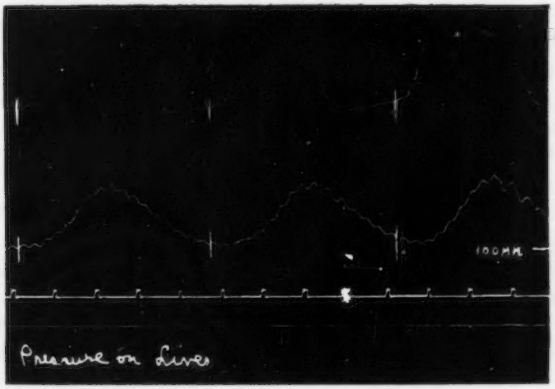

Fig. 2. Effect of pressure on liver. Dog, weight 12.5 kilos. Animal in apnea. Upper curve, pressure recorded by balloon between diaphragm and liver, connected to Marey tambour. Upstroke denotes application of pressure to liver. Lower curve—carotid blood pressure. Time in seconds. Note latency in effect.

Reviewing all the effects of respiratory movements upon the venous supply to the right heart, it is evident that all the factors but one tend to augment that supply during inspiration. The lowered intrathoracic pressure, the effects of increased abdominal pressure on the abdominal vessels, the pure movements themselves, all tend to increase the flow of blood to the thorax. The single factor, not of very much moment in comparison with the others, tending to decrease the flow, is the effect of increased abdominal pressure in hindering venous return from the lower extremities. Therefore, one may conclude that the resultant of all the forces acting upon the venous return to the heart will tend to produce

an increase in that return and consequently an increased cardiac output with a rise in arterial pressure.

Historically, an important point in the discussion of the respiratory wave has been the effect of nervous factors. In fact, no longer ago than 1915, Snyder (31) asserted that the wave was largely, if not entirely a phenomenon of vagus inhibition due to what he termed an "irradiation" stimulus from the respiratory center. He published a blood pressure tracing of a period of apnea produced by stimulation of the central end of the cut vagus in which he found after the stimulation was suspended, a respiratory wave before respiratory movements began. His procedure was repeated on ten dogs more than one hundred times using every possible variation in the strength of current for stimulation and similar findings to his were never obtained.

Henderson and Barringer (8) also sought to invoke the interference of nervous factors in the production of the respiratory wave. They believed that other factors were incompetent of explaining the phenomenon and so called attention to the inspiratory quickening of the heart rate which is often seen in blood pressure tracings from man and animals, ascribing the variations in pressure to those changes in rate. Many other workers have held similar opinions.

It was shown many years ago by Einbrodt (32) that section of the vagi in animals eliminated the variations in pulse rate without altering the wave in the blood pressure. This, of course, does not prove that there are no nervous factors entered in for it is conceivable that the sympathetics might alter the force of the heart beat without altering its rate. However, recording arterial pressure tracings on naturally breathing animals whose vagi were cut and stellate ganglia removed (see fig. 3), showed respiratory waves with no diminution of size and with normal sequence, proving that the nervous connections of the heart are not essential to the production of the respiratory wave.

Mathieu (33) has contributed an interesting piece of evidence against the idea that nervous influences play any large part, viz., that in young dogs it is impossible to demonstrate any anatomical connections between the respiratory and cardiac centers in the medulla, and yet there is a definite respiratory wave. He also pointed out that only a small proportion of blood pressure tracings show any arythmia while almost all show the respiratory wave. It might be noted here that of the sixty dogs used in the experimental work on which this paper is based, only two showed any respiratory arythmia and these only toward the end of the experimentation period when there was probably a partial asphyxia.

In connection with the respiratory arythmia it should be noted that it is always observed after the period of falling pressure, and as such may be explained as a compensatory mechanism, attempting, in conformance

Fig. 3. Effect of extirpating the stellate ganglia and cutting the vagi on the respiratory wave. Dog, weight 10 kilos. Normal respiration. Upper curve, intratracheal pressure, mercury manometer. Lower curve—carotid blood pressure. Time in seconds. Showing respiratory wave on denervated heart.

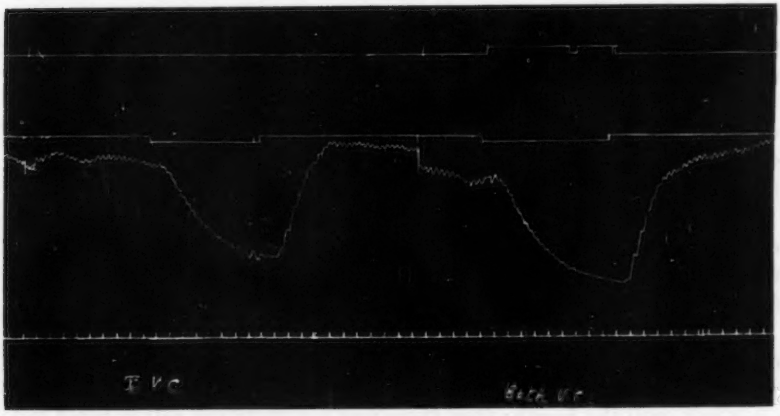

Fig. 4. Effect of clamping venae cavae on arterial blood pressure. Dog, weight 10 kilos. First and second lines—marker indicates application of clamp to vein. Third line—carotid blood pressure. Fourth line—time in seconds. Zero level of time line. First fall = clamp inferior vena cava. Second fall—clamp inferior and superior vena cava. Note latency in fall of arterial pressure.

to Marey's law, to counteract the fall in blood pressure due to decreased supply by an augmented rate. This view finds support in the fact that when the vagi were cut in an animal showing arythmia, there was a sharpening of the peaks and falls in the respiratory wave. This procedure was only carried out on one animal so the data are inconclusive and the explanation can only be given as a possibility.

It is very evident, however, that although the nervous regulation of the heart beat is sometimes concerned in the respiratory cycle, it is not the paramount factor in the production of the variation in arterial pressure.

CORRELATION OF THE VARIOUS FACTORS AT WORK. TIME RELATIONS. OF THE CAUSE AND EFFECT IN THE RESPIRATORY WAVE: Having come to a conclusion regarding the factors concerned in the respiratory wave and the qualitative, if not quantitative, effect of each, it remains to correlate them in such a way as to fit the picture we see in the facts at hand. It was noted before that an outstanding feature in the literature of this subject was the inability of various observers to agree even on the phase in the respiratory cycle in which the rise and fall in blood pressure occurred. It became a question, therefore, whether the problem might not be settled by a study of the time relations of the changes taking place. It seemed to have been quite generally assumed by workers on the problem that a change occurring in a given phase of respiration should show its effects in that phase. It appeared plausible, however, that there might be a delay in those effects sufficient to explain the disagreement in observations which was so apparent. Therefore, a series of experiments was conducted to determine how soon changes in the venous supply to the right atrium were reflected in the systemic arterial pressure.

The first method pursued was that of clamping the inferior or superior vena cava, or both, at a given moment, automatically recorded on the arterial pressure trace by means of a signal magnet in circuit with the hemostat used in the work and noting the effect on arterial blood pressure. The hemostat was equipped with an insulated post so adjusted that a contact was made when the clamp was fixed. By this means it was possible to tell accurately how many heart beats intervened between the clamping and the change in blood pressure.

It was found when the inferior or superior vena cava of an anesthetized dog, in a condition of apnea, was clamped near the heart, that the arterial pressure curve (fig. 4) showed no change before the third heart beat, often not until the fourth; then there was a continuous, rather rapid fall to the level which could be maintained by the remaining sources of supply. When the clamp was released there was again no evidence of it for a period of from two to four heart beats after which there was a rapid rise to the former level. This procedure was repeated so many times with exactly similar results that there seemed to be no doubt at all as to the correctness

of the observations. In certain experiments both the inferior and superior venae cavae were clamped and in these cases, too, there was no evidence of the change shown on the arterial pressure curve till three heart beats after the clamping. Figure 4 shows the effect on arterial blood pressure of clamping the inferior vena cava; and the effect of clamping both inferior and superior vena cava simultaneously; figure 5 the effect of clamping them after tying off the azygos veins.

In some experiments where both the venae cavae were clamped it was found that the blood pressure only fell to between one-third and one-half of its original level. It was found that when they were clamped after ligating the azygos vein, the fall was practically complete, thus indicating that under certain conditions the azygos veins are important factors in carrying blood to the heart.

These experiments were repeated on animals with denervated hearts to eliminate any possibility of a reflex mechanism obscuring the effects of the interference with heart supply. No difference was observed.

The inference from these results is obvious. They mean that one need not look to the systemic arterial pressure for evidences of changes of inflow to the right heart for at least three beats after the change has occurred.

Therefore, one would not expect to begin to see the effects of inspiration upon cardiac supply reflected in the arterial pressure until the third heart beat of that phase. Likewise, the effects of expiration would appear about three beats after that phase begins.

The results obtained with clamping the great veins are very suggestive but they do not prove that such a condition exists in normal respiration. It remained to record accurately the changes in intrathoracic pressure and the arterial pressure in order to prove the point. In a normally breathing animal it is obviously impossible to record these changes because the phases are changing so rapidly that it would be impossible to obtain a pure effect of inspiration, for example, before expiration had set in and obscured its real effect. So the expedient of curarizing the animals and using artificial respiration was adopted. Two tubes were inserted through the chest walls into the pleural cavities; one connected with a manometer, another with a source of suction. In this way it was possible, after stopping artificial respiration, to produce inflation of the lungs by lowering the intrathoracic pressure. They could easily be collapsed by increasing the pressure to the atmospheric. The advantage of the procedure lay in the fact that the inspiratory or expiratory condition could be maintained as long as it was necessary or desirable in order to show the pure effects of each phase, or the resultant condition when the pressure was raised and lowered in a sequence resembling that of normal respiration. Figure 6 shows the effect of a lowered intrathoracic pressure. At the beginning of the tracing the pressures were maintained long enough to get the

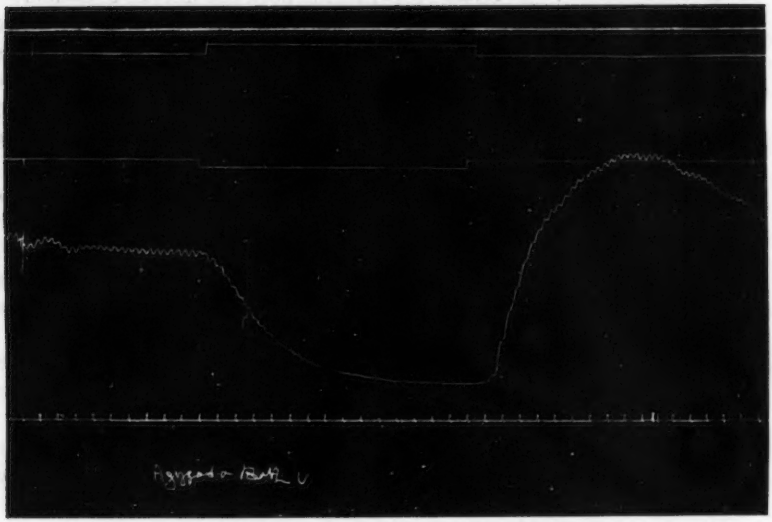

Fig. 5. Effect of clamping superior and inferior venae cavae after ligating azygos. Dog, weight 10 kilos. First and second lines—marker indicates application of clamps to veins. Third line—carotid blood pressure. Fourth line—time in seconds. Note latency in fall of pressure.

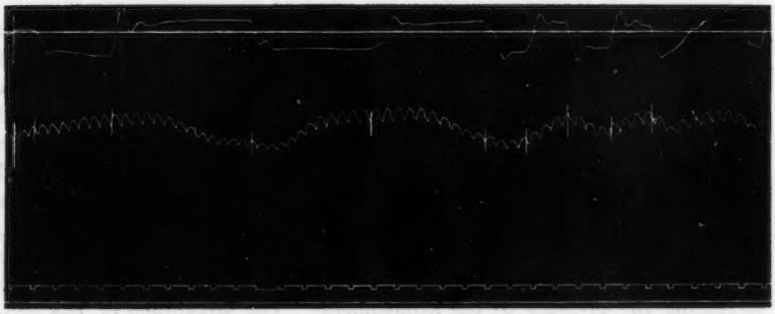

Fig. 6. Effect of artificial variations of intrathoracic pressure. Dog, weight 12.5 kilos. Animal in apnea. Heart denervated. Upper curve—variations in intrathoracic pressure recorded by mercury manometer. Downstroke—increase in negative pressure. Straight line marks intrathoracic pressure (about 750 mm.) in state of respiratory quiet. Lower curve, carotid blood pressure. Time in seconds.

pure effects of reduced or increased pressure. There is a period of three heart beats when there is no significant change, if anything, a slight fall, then a rapid and considerable rise continuing as long as the low pressure is maintained. When the pressure is raised there is again a period of several heart beats before the fall in pressure begins. This continues rapidly to the level it will maintain, tending to rise again slowly from this level.

Thus, inspiration is seen to cause a slight preliminary fall due to the effect of impeded pulmonary circulation before the effect of the increased supply to the right side of the heart has a chance to affect the systemic arterial pressure. Expiration, after a preliminary slight rise, causes a great fall. The rise is due to the freer passage of blood through the lungs in the first beat or two of expiration and the fall is due to the decreased supply to the right heart.

The next step was to see what results would be obtained when the intrathoracic pressure was varied in about the rhythm of normal respiration. As the last part of figure 6 shows, the effect of inspiration is seen in expiration and the effect of expiration in inspiration. In other words, the latent period of the changes is so great that there can be a complete reversal in the time relations between cause and effect. When there are six heart beats or less, per respiration, there will always be a fall during the inspiration, and a rise during expiration. The first effect of inspiration, the slight fall, will come on while the expiratory fall is still going on, then, on the third beat after the beginning of inspiration, there is usually the rise. This comes on in time to show up during the expiratory phase. So expiration is the period of rising pressure when the heart respiration ratio is about 6:1. During expiration the real effects of inspiration begin to appear. As the number of heart beats per respiration increases, the effects of the various phases tend to come on before the end of the phase is reached. With an 8:1 ratio, the inspiratory rise is just under way before expiration begins. That ratio is a frequent one in dogs under morphine and ether, and there one finds the rise coming on just at the end of inspiration, as one would expect. Figure 7 shows the relations of the respiratory variations at this ratio in a normally breathing dog.

As the number of heart beats per respiration decreases below six the respiratory variation tends to diminish because of the superposition of antagonistic forces upon each other. This, also, is well in agreement with observed facts. Figure 8 shows a tracing with a heart respiration ratio of 3:1, and figure 9 a ratio of 4:1. The variations in arterial pressure are seen to be slight.

As long as the absolute heart rate remains constant, the heart respiration ratio seems to be the only factor that affects the time of appearance of the respiratory variations in arterial pressure in relation to the phases

Fig. 7

Fig. 8

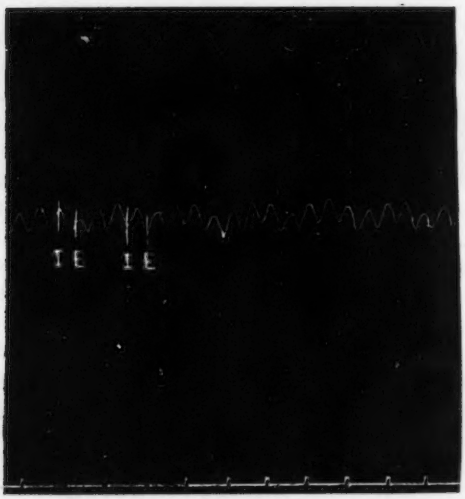

Fig. 9

Fig. 7. Respiratory wave with heart respiration ratio 8:1. Dog, weight 9 kilos. Vagi cut. Animal breathing naturally. Upper curve—respiration recorded by Hg manometer connected with pleural cavity. Downstroke—inspiration. Lower curve—carotid blood pressure. *E*—beginning of expiration. *I*—beginning of inspiration. Time in seconds. Rise commencing just before expiration begins.

Fig. 8. Normal respiratory wave with heart respiration ratio 3:1. Dog, weight 9 kilos. Normal respiration. Upper curve—respiration recorded by Hg manometer connected with pleural cavity. Downstroke—inspiration. Lower curve—carotid blood pressure. *E*—beginning of expiration. *I*—beginning of inspiration. Time in seconds. Practically no respiratory wave.

Fig. 9. Normal respiratory wave with heart respiration ratio 4:1. Dog, weight 8 kilos. Normal respiration. Upper curve—respiration recorded by Hg manometer connected with pleural cavity. Downstroke—inspiration. Lower curve—carotid blood pressure. *E*—beginning of expiration. *I*—beginning of inspiration. Time in seconds. Rise in expiration.

of respiration. However, as the absolute heart rate increases, the force of each beat tends to decrease because there is less blood to act upon. Therefore, the absolute number of heart beats between the beginning of inspiration and the resultant rise in blood pressure is increased when the heart rate is rapid. The depth of respiration, that is, the extent of change in pressure within the thorax, is also a factor to be considered; the greater the depth of respiration, the greater will be the variation in arterial pressure and the sooner it will counterbalance the effects of the last phase and establish its own effect.

SUMMARY

1. It appears probable from the evidence available that the respiratory wave in arterial blood pressure is the resultant of a number of factors affecting the output of the heart. Most important is the lowered intrathoracic pressure facilitating the flow of blood to the atria of the heart. Secondary in importance is the effect of the condition of the lung vessels on the flow of blood from the right to the left side of the heart. That effect is operating largely in opposition to the first named force, but is largely overshadowed by it in conditions of natural breathing.

2. The impression, current in textbooks of physiology, that the inner and outer layers of the lung wall are pulled apart by the negative pressure within the thorax in inspiration, is entirely erroneous. The only force operating is that of atmospheric pressure acting against the elastic tension of the lung, therefore the two layers of the lung must be pushed out in the same direction. It follows, therefore, that the lung vessels will be stretched in length and decreased in bore, thereby increasing the resistance to the flow of blood in the distended condition.

3. Changes in the rate and force of the heart through its nervous control are not fundamentally responsible for the respiratory wave.

4. Changes in the venous supply to the heart are not simultaneously reflected in the arterial pressure curve. Three heart beats usually intervene between the alteration in the inflow and the change in the arterial pressure.

5. It was found that inspiration by lowered pressure within the thorax caused a rise in blood pressure after a delay of from two to four heart beats. Expiration by raised pressure caused a fall after a similar delay. It is evident, therefore, that the inspiratory act produces a rise in blood pressure and the expiratory act a fall. In which phase of respiration the rise or fall appears is dependent upon the heart respiration ratio. If that is large, the effects of a given phase will come on before its end; if it is the ordinary ratio for man, 6:1, the effect of one phase will show up during the next; if it is very small, there will be a superposition of antagonistic forces and the wave will almost disappear.

6. In the analysis and interpretation of respiratory changes in arterial blood pressure, one is not justified in assuming that simultaneous factors are responsible for those changes. The latent period in the process, that is, the time between the respiratory movement and the appearance of the effects of it on the blood pressure curve, must be taken into account.

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PERMEABILITY OF THE URINARY BLADDER TO UREA AND SODIUM CHLORIDE

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There are few subjects in physiology that have a larger and more controversial literature than that of absorption from the urinary bladder. For the literature the papers of Garota (1), Hamburger (2), and the quite recent one of Mann and Magoun (3) may be consulted. We shall refer only to the more important communications dealing with the permeability for substances normally present in the urine.

In 1856 Kaupp (4) studied the differences seen in specimens of urine retained in his bladder for a period of 12 hours and in specimens consisting of 12 hourly voidings under conditions of constant fluid intake, activity and diet. He found that over a period of 60 days the two series of specimens showed a fairly constant difference, the retained specimen was of smaller volume and contained less urea, sodium chloride, phosphates, and sulphates than that obtained by hourly voiding.

Treskin (5) using dogs found definite changes in the specific gravity, ash, chlorides and urea content of the urine introduced into the bladder and allowed to remain for three to four hours.

Ashdown (6) found that a 6 per cent solution of urea left in the bladder for 5½ hours showed a decrease of 3.75 per cent in volume and 10.5 per cent of urea; another experiment with 7 per cent urea over a period of 6 hours showed a decrease of 4.6 per cent volume and 19.4 per cent urea.

Morro and Gäbelein (7) using dogs and a period of 3½ hours found no adsorption with 2 per cent urea; using glucose solutions of 5 to 50 per cent over periods of 3 to 4 hours, they found an increase in the volume of the fluid instilled and a decrease in the specific gravity. These changes varied in degree with the per cent of glucose introduced.

Garota (1) used a method of withdrawing a portion of urine from the bladder with a syringe and needle at various intervals after ligation of the ureters and concluded that the bladder was slightly permeable to the normal urinary constituents.

Cohnheim (8) concluded from his study of the question that the bladder was absolutely impermeable to the normal urinary constituents in concentrations ordinarily encountered, but that the use of higher concentrations or injury to the bladder epithelium allowed permeation. This conclusion of Cohnheim has been frequently accepted, although his experiments are not entirely convincing.

Shoji (9) has quite recently reinvestigated the subject. Working with rabbits, he studied the behavior of sodium chloride solutions varying from 0.25 to 4.0 per cent. Hemoglobin was added to the solutions before placing them in the bladder, and the change in the concentration of the hemoglobin taken to indicate the water exchange

through the bladder wall. With a 1 per cent solution of chloride no change in the water or chloride was observed; with solutions of 2 and 4 per cent water passed in and salt out of the bladder; with 0.5 per cent solution water passed out of the bladder but no movement of salt was observed; with 0.25 per cent solution, salt passed in and water out of the bladder.

Rabbits were used in all of our experiments. They were anesthetized with urethane supplemented with ether during the operative procedure. In most of the experiments a soft rubber catheter was introduced into the bladder, the penis tied around the catheter, and the ureters doubly ligated and cut through a middle abdominal incision. In certain other experiments a different procedure, which is described later, was used. The bladder was emptied and washed several times with the fluid to be introduced. The solution was warmed to body temperature, and allowed to flow slowly through the catheter which was then clamped. Forty cubic centimeters of fluid was usually introduced. After an appropriate time, usually one hour, the catheter was opened to allow the fluid to flow into a graduated cylinder. Analyses for urea and sodium chloride were made upon the solution before and after its introduction into the bladder. The figures for fluid volume usually showed very slight changes, but the method for water exchange is not sufficiently accurate to warrant their discussion. Duplicate analyses were made for chlorides by the Volhard procedure and triplicate ones for urea by the urease method.

The following condensed protocols illustrate experiments in which solutions of the same concentration were introduced in successive periods.

Experiment 8. Solution 0.3 M sodium chloride and 0.05 M urea. Periods of one hour. Concentrations in milligrams per cubic centimeter.

NaCl			UREA		
Before	After	Per cent change	Before	After	Per cent change
18.1	17.8	−1.6	2.93	2.94	+0.35
17.8	17.5	−1.7	2.91	2.91	0.00
17.8	17.7	−0.55	2.92	2.92	0.00
17.8	17.7	−0.55	2.91	2.92	+0.35
Average.....		−1.11			+0.18

Experiment 9. Solution 0.5 M sodium chloride and 0.05 M urea. Periods of one hour. Concentrations in milligrams per cubic centimeter.

NaCl			UREA		
Before	After	Per cent change	Before	After	Per cent change
30.0	25.8	−14.0	2.93	2.58	−13.64
29.9	26.0	−13.3	2.88	2.49	−13.52
29.8	25.2	−15.0	2.95	2.53	−13.60
29.7	25.3	−14.7	2.92	2.54	−13.00
Average.....		−14.2			−13.4

With 0.5 M sodium chloride there is quite a marked change in the concentration in one hour, and an equally marked change in the concentration of urea. With the 0.3 M sodium chloride there seems to be a slight decrease in the concentration of the salt, but no detectable change in the urea. Sometimes the change in the salt in this solution was less marked. The following experiment is typical of several carried out with different concentrations of sodium chloride.

Experiment 14. Periods of one hour. Concentrations in milligrams per cubic centimeter. The urea was 0.05 M.

MOLAR	NaCl			UREA		
	Before	After	Per cent change	Before	After	Per cent Change
0.4	23.7	23.4	−1.0	2.84	2.87	+0.95
0.5	29.2	28.6	−2.0	2.94	2.82	−4.0
0.6	35.7	32.6	−8.9	2.89	2.62	−9.0
0.7	40.4	34.4	−15.5	2.91	2.42	−13.4

The change in concentration of the salt is greater, the greater the original concentration of the solution used. Although urea is of quite a low and constant concentration the amount by which it changes after its stay in the bladder appears to be influenced by the concentration of the salt. That the changes in the concentration of the salt and urea in the fluid after its stay in the bladder signify actual absorption of these substances and not only a transfer of water to the bladder fluid is shown by a consideration of the changes in the volume of the fluid. Although, as before stated, these could not be accurately determined, the figures quite clearly indicate that the change is not of sufficient magnitude to account for the change in concentration. That an actual absorption of urea and salt takes place was determined by experiments in which the total quantity of these substances introduced was estimated, and the total quantity recovered in the fluid and washings obtained from the bladder determined.

Experiment 18. Different concentrations of sodium chloride in the bladder for periods of one hour. Figures represent total amounts introduced and recovered in milligrams.

MOLAR	IN	OUT	PER CENT ABSORBED
0.3	436	434	0.4
0.4	586	571	2.6
0.5	721	692	4.0
0.6	885	806	8.9
0.7	1003	874	12.9

Experiment 19. Solution of urea 0.5M for periods of one hour. Figures represent total amounts in milligrams introduced and recovered.

IN	OUT	PER CENT ABSORBED
1182	1130	5.2
1182	1090	7.8
1182	1100	7.0
1182	1115	5.7
Average.....		6.4

Solutions of 0.4 M or stronger are certainly more concentrated than the maximum concentration of sodium chloride which can occur in rabbit's urine even after the administration of salt. The rapid absorption from these solutions may therefore be due to injury to the bladder epithelium. Experiments to test this point were carried out with 0.7 M solution by determining the change in concentration of a 0.3 M solution before and after the stronger solution had been used. In four out of six experiments of this type a definite increased absorption of the 0.3 M solution was found after the stronger solution had been used.

On account of the possibility of injury from even the weaker concentrations and on account of the fact that the absorption of one substance seems to be influenced by the concentration of another, we have carried out two series of experiments with the animal's own urine. In the first of these, the procedure was exactly similar to that used with the above solutions. The urine was always obtained from the animal just before the experiment was started. Table 1 gives the results. In experiments 25, 27, 28 and 29 the rabbits were given salt by mouth before the experiment. The urine remained in the bladder for three hours. All concentrations are molar.

That an absorption, or at least a change in concentration, of urea and sodium chloride takes place when urine is allowed to remain in the bladder for three hours seems evident from these experiments. Two objections to the technique, however, may be advanced. The use of a catheter may injure the bladder mucosa, and the loss of carbon dioxide from the urine may in some cases increase its alkalinity sufficiently to do the same. A second series of experiments was performed in an attempt to meet these objections. Rabbits were kept under observation until it appeared that the bladder contained more or less urine. They were then anesthetized, the ureters exposed extraperitoneally by incisions through the flanks, and doubly ligated and cut. A sample of urine was then obtained from the bladder by exerting gentle pressure on the abdomen, and a second sample removed after a period of three hours. Table 2 gives the results of the experiments. In experiments 32, 33

and 34, the animals received salt before the experiment. All figures represent molar concentrations.

These experiments which, as far as we can see, are entirely free from objections, definitely prove that the urine changes in composition during its stay in the bladder. The figures indicating the percentage change in concentration vary widely, and the amount of change appears to depend partly on the total osmotic pressure of the urine as well as on the concentration of the substance in question. When the concentration of the substance is very low and the total osmolar concentration is

TABLE 1

EXPERIMENT NUMBER	NaCl			UREA		
	Before	After	Per cent change	Before	After	Per cent change
19	Trace			0.536	0.528	−1.5
20	Trace			0.336	0.326	−3.0
21	0.083	0.086	+3.6	0.319	0.314	−1.6
25	0.206	0.186	−10.0	0.477	0.363	−25.5
26	Trace			0.403	0.383	−5.0
27	0.023	0.038	+65.0	0.257	0.241	−6.2
28	0.149	0.138	−7.3	0.582	0.562	−3.7
29	0.330	0.294	−10.9	0.590	0.526	−11.0

TABLE 2

EXPERIMENT NUMBER	NaCl			UREA		
	Before	After	Per cent change	Before	After	Per cent change
32	0.241	0.217	−11.2	0.268	0.168	−37.3
33	0.175	0.168	−4.0	0.085	0.085	0.0
34	0.135	0.134	−0.7	0.028	0.0275	−1.7
35	Trace			0.341	0.337	−1.2
36	Trace			0.317	0.310	−2.2
37	Trace			0.960	0.907	−5.5

not high little or no absorption can be demonstrated. The marked absorption in experiment 32 as well as that of experiment 25 in table 1 may be due to injury from the amount of salt given before the experiment. In these experiments about one-half of the quantity of sodium chloride which we have found to be fatal to rabbits was given to increase the concentration of the urine. Of course, the slight changes in the concentration of salt and urea in these experiments do not prove that these substances are absorbed from the bladder since the changes could be entirely explained by a diffusion of water into the bladder. But in view of the fact that we have shown similar changes in the case of pure solu-

tions of urea and sodium chloride to mean some absorption of these substances, it seems reasonably certain that the changes can be so interpreted here.

The slight permeability of the bladder to the normal constituents of the urine would not seem to serve any physiological function, but to be rather an interference with its normal function as a temporary receptacle for the urine. This permeability must, therefore, probably be regarded as a defect in the organ; the bladder is not quite perfect as a mechanism for preventing osmotic exchange between the urine and the body fluids.

SUMMARY

1. Solutions of sodium chloride from 0.3 to 0.7 molar containing 0.05 molar urea when allowed to remain in the rabbit's bladder show a diminution in the concentration of salt which is greater with the higher concentrations. The decrease in the concentration of urea is greater in the solutions with the higher salt concentration.

2. A determination of the absolute quantity of salt or urea in these solutions indicates that an absorption of these substances has taken place.

3. Changes in the concentration of sodium chloride and urea in the urine can be demonstrated during its stay in the bladder.

4. Since these changes in concentration take place where injury to the bladder seems to be excluded, the conclusion is drawn that the bladder wall is normally slightly permeable to these substances.

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THE EFFECTS OF ADRENALIN ON THE REACTION OF INTESTINAL SEGMENTS TO OXYGEN

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Attempts to deduce the physiological rôle of the adrenal glands from studies on the pharmacology of adrenalin have been notably unsuccessful. While the phenomena resulting from intravenous injections of this drug can be harmonized with the "emergency theory" of adrenal function they offer no consistent explanation of the significance of the glands during periods of ordinary quiet existence. The evidence on this point has been considered elsewhere (1). Of recent years the trend among physiologists has been to assume that it is the cortex of the suprarenal, and not the adrenalin-yielding medulla that plays the essential rôle. The arguments leading to this conclusion are especially three. The amount of the drug required to cause apparent changes in organ functions is in general greater than that which, according to best available evidence, the gland is able to produce (2). It is also urged that it is the cortex and not the medulla that is essential to life, but the possibility is still open and supported by some evidence that the cortex produces adrenalin (3). That an animal can remain in apparently good health with the adrenals denervated and the output of adrenalin reduced below the pharmacologic threshold is also cited. In the case of the thyroid, denervation, as is well known, introduces no harmful diminution in secretion and the same may be true of the adrenals. That no adrenalin has been detected in the blood leaving the denervated adrenals may be merely a special instance of inadequacy of the technic to demonstrate lesser quantities of this substance. The problem is as yet by no means settled.

It is quite possible that the adrenals may play an essential rôle by affording a constant supply of adrenalin which is necessary for normal metabolism. Burridge (4) has shown, for instance, that the addition of a slight amount of this substance markedly improves the activity of a heart beating in unbalanced Ringer's solution. More recently, convincing evidence has accumulated that minute quantities lead to augmented basal metabolism (5), i.e., to increased tissue oxidation. That this augmentation is due to a catalytic effect of the adrenalin is indicated

both by the minuteness of the amount involved and by the results of direct experimentation (6) in which it has been shown that the systemic reaction to the drug is not diminished by passing it through a fatigued muscle, the activity of which is thereby, nevertheless, notably increased.

METHODS. If adrenalin significantly facilitates the oxidative reactions it should improve the activity of tissues under conditions in which low oxygen tension is a limiting factor. An investigation of this possibility has afforded the data herein reported. Some of the results have previously been published in preliminary abstract form (7). We first investigated the suitability of the frog's heart for such a study. But it was found that the effect of partial asphyxia was relatively slow in appearing and that satisfactory variations in the degree of asphyxia were difficult to secure and to control. Segments of small intestine were then selected. As is well known, adrenalin ordinarily produces marked depression of the spontaneous contractions and tonus in such preparations. Several observers, however, have noted augmentation when very dilute solutions were used. Schafer, alone, so far as we are aware, has worked with preparations that gave only augmentation with any effective concentration (8). Peristalsis in the intact animal can readily be shown to be augmented as a secondary reaction to injections causing a primary depression (9). Our experience in this study has been that higher concentrations always lead to depression of the intestinal activities.

This fact offers one of the major difficulties in such an investigation as that herein reported. If too much of the drug is used the primary depression completely surpasses any other effect that might tend to occur. On the other hand, if the solution is too dilute, no effect at all is elicited. Another aspect of the difficulty is that the thresholds, of both the augmentation and the depression, vary from one preparation to another and progressively change in case of any given preparation. Success was most often achieved when the concentration was approximately one part in a hundred millions.

The methods employed have been previously reported (10). In brief, segments of small intestine of rats were suspended from light writing levers in 50 cc. beakers of mammalian Ringer's solution of pH 8. The beakers were surrounded by a large volume of water maintained with sufficient constancy at approximately 37°C. A considerable number of the segments proved to be so erratic in their reactions as to be useless for comparative studies. But others gave sufficiently similar reactions from one stimulation to another as to permit detection of the influence of an introduced variable. Such preparations characteristically went through an evolution of reaction patterns, but this was of such slow progress as to introduce no confusion in the interpretation of experimental results. It should be emphasized that each individual segment

that is used should be studied individually and proved to be regular in its reactions before it is employed as a test object. Oxygen was administered by passing it through a small bore tube opening beneath the intestinal segment; it escaped in small bubbles at a rate of about one hundred a minute. When administered more slowly the rate affected the reaction but in case of more rapid bubbling the rate did not have to be controlled with absolute accuracy. Sufficient uniformity was secured by keeping the pressure constant and sending the oxygen alternately through the beaker and through a waste valve. In the earlier period of the study the importance of the stimulating effect of the agitation of the surrounding fluid by the escaping bubbles was underestimated and the reactions to the oxygen as such and to this mechanical influence were not separately considered. Later the preparations were stimulated alternately by oxygen bubbles and by rapid vibrations transmitted from the "buzzer" of a Harvard inductorium to the thread by which the seg-

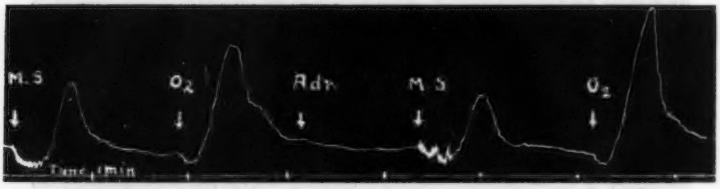

Fig. 1. Longitudinal segment of ileum from female rat weighing 120 grams. At points marked *M.S.*, segment stimulated mechanically for 20 seconds. At point *Adr.*, adrenalin chloride added to concentration 1:60,000,000. At points marked O_2, oxygen bubbled through fluid for 10 seconds. (Reduced ⅓.)

ment was attached to the writing lever. The increment of the reaction to the oxygen bubbles as compared to that resulting from the vibrations was interpreted as due to the oxygen per se.

The trend of the results is exemplified in the graph reproduced as figure 1. This was obtained from a freshly prepared 2 cm. segment of the upper ileum of a female rat weighing 120 grams. The appearance of the uterus indicated that the animal was not in estrus. The preparations had been stimulated at intervals of three minutes for a period of two hours and ten minutes and was at a stage of slowly and regularly diminishing consecutive reactions. It was stimulated alternately by the "buzzer" for twenty seconds and by oxygen for ten seconds. Adrenalin chloride (Parke, Davis & Co.) was then introduced to give a concentration of one part in sixty millions. This caused no immediate change except a very slight augmentation of tonus. The mechanical stimulation and the oxygen administration were then repeated. The reaction to the oxygen was materially increased. In this case, the reaction to

the vibrations, which had been slowly decreasing, continued to decline. In some other instances, however, the reaction to mechanical stimulation were slightly augmented after the adrenalin was introduced. The augmenting influence of the adrenalin was still apparent after the lapse of eight minutes.

The most striking example secured of adrenalin augmentation of the reaction to oxygen is shown as figure 2. A segment 1.5 cm. long was

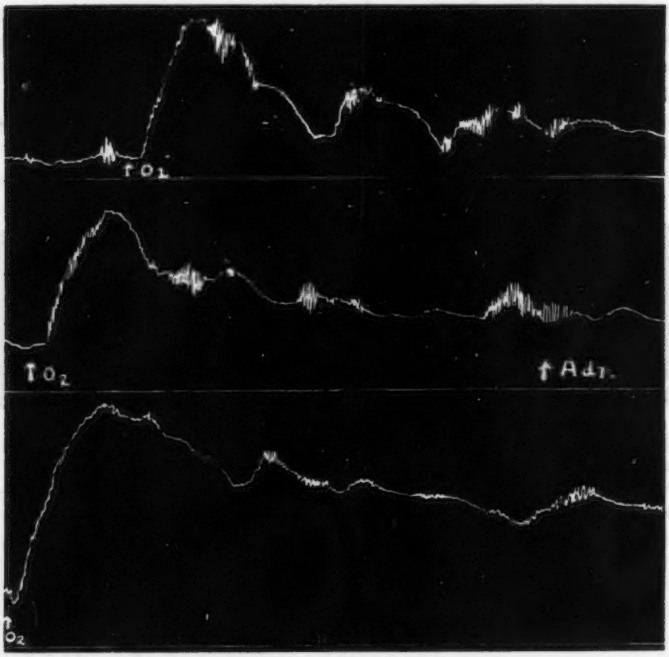

Fig. 2. Longitudinal segment of ileum from adult male rat; kept 6 hours at 0°C. before use. Graphs show three consecutive reactions to oxygen administered by bubbling for 30 seconds. At point *Adr.*, adrenalin chloride added to concentration of 1:120,000,000.

taken from the ileum of an adult male rat. The intestine had been kept for six hours in a moist chamber (covered beaker) surrounded by ice-water. After thirty minutes' suspension in Ringer's solution and three administrations of oxygen for periods of thirty seconds each, the preparation gave two closely similar reactions to the oxygen. Adrenalin chloride was then introduced to give a concentration of 1:120,000,000. This had no detectable influence on the unstimulated preparation, but the

succeeding reaction to oxygen was about one-third higher than before and was partially sustained as a condition of heightened tonus for a period of more than ten minutes. At the end of thirteen minutes the reaction to oxygen was approximately equal to the control reaction. The introduction of more adrenalin to give a concentration of 1:60,000,000 resulted in a decrease of the reaction to about one-third. This experiment is open to the criticism that the preliminary applications of oxygen were not numerous enough to demonstrate the suitability of that particular preparation for use as a test object. The augmentation of the reaction, however, was greater than any that appeared spontaneously in these studies, and the results of later administrations of oxygen bore out the assumption that the segment in question was one of the type giving steady reactions.

Discussion. The fact that the intestine reacted more vigorously to oxygen after the application of adrenalin might be interpreted as an example of summation of stimuli, one of which was inadequate. In view, however, of our ignorance of the ultimate nature of stimulation in any case, such an explanation rather begs the question. Distinctly more enlightening is the assumption that not only in the case herein reported, but also in case of the facilitation of contractions of skeletal muscle and of the augmentation of basal metabolism, the essential nature of the phenomenon is catalysis of tissue respiration. Presumably more light would be thrown on the problem by further investigation of oxygen consumption by quiescent tissues before and after the application of adrenalin. The results of Gruber and others (6), (11) on mammalian muscle as compared with those reported by Griffith (12), Eddy (13) and others on frog's muscle indicate that tissues of the higher animals would be more likely to give clean-cut results.

SUMMARY

1. The reaction of rat intestine to oxygen was investigated before and after the application of adrenalin.

2. With suitable preparations intestinal segments in saline solutions of adrenalin react more vigorously than in saline alone.

3. The most effective concentration of adrenalin was found to be approximately 1:100,000,000.

4. More concentrated solutions depress activity and weaker solutions are without detectable influence.

5. The augmented reactions in the presence of adrenalin are ascribed to catalysis of tissue oxidation by this substance.

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STUDIES ON BIOLUMINESCENCE

XVI. What Determines the Color of the Light of Luminous Animals?

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The light of different luminous organisms may vary through all spectral tints from red to violet. Yellowish, greenish, or bluish hues predominate. In most cases the color is due to a definite spectral band of light emitted during the oxidation which underlies light production, but in a few cases the color is due to color filters over the luminous organ. I am not concerned in this paper with colors due to absorption filters but only with those resulting from some peculiarity of the oxidative mechanism. What determines the color of the light emitted during the oxidative process?

For light production, an oxidizable body, luciferin, is oxidized in the presence of an enzyme-like body, luciferase (1). These substances cannot be demonstrated in all luminous animals, but it is possible to demonstrate them in the fire-flies, in *Pholas*, a mollusc, in ostracod crustacea, and in *Odontosyllis*, a worm. The luciferin of one of the groups above named, when mixed with the luciferase from another group, does not react with light production, but the luciferin from a member *within* one of the groups (a different genus or species) will react by light production with luciferase from another member within the group. Thus, *Cypridina* luciferin and *Pyrocypris* (another ostracod) luciferase will produce light when mixed, and vice versa, but *Cypridina* luciferin will not produce light with fire-fly luciferase or *Pholas* luciferase or *Odontosyllis* luciferase, and vice versa (2).

Some years ago (3)[1] I found that if one mixed luciferin from a fire-fly having a yellowish[2] light, *Photuris pennsylvanica*, with the luciferase of a fire-fly having a reddish[2] light, *Photinus pyralis*, the resultant light would be reddish. With *Photinus* luciferin and *Photuris* luciferase the light was yellow. Hence the insect supplying the luciferase determines the color of the light.

[1] The term photophelein is used for luciferin and photogenin for luciferase in this paper.

[2] These tints are due to actual differences in the spectrum of the two fire-flies (4).

The conclusion drawn from this fire-fly experiment does not appear unequivocal to me for the following reason. Luciferase extract is prepared by grinding the photogenic cells with water and allowing the filtered extract to stand until the luminescence disappears and the extract becomes dark, indicating that the dissolved luciferin has been oxidized.[3] During the process of grinding, some photogenic cells or photogenic granules may remain intact. If the luciferin solution (prepared by extracting photogenic cells with boiling water, which destroys luciferase but not luciferin) contains a cytolytic agent these photogenic cells or granules may be cytolyzed, with liberation of fresh luciferin previously bound in some way in the extract of luciferase. The color of the light resulting would naturally be that of the fire-fly supplying the luciferase extract which still contains some unoxidized luciferin.

This cytolysis[4] of photogenic granules is a marked phenomenon in the coelenterates. A production of light may readily be obtained from sea water extracts of the luminous medusae, *Aequorea* or *Mitrocoma*, allowed to stand until the luminescence has disappeared, by simply adding distilled water or cytolytic agents such as chloroform, saponin or sodium glycocholate (5), (6). These forms do not give the luciferin-luciferase reaction. The fire-fly may present cytolytic luminescence effects superposed on the luciferase-luciferin reaction. Consequently it is important to have confirmatory evidence as to the color of luminescences resulting from "crossing" of luciferins and luciferases of different species, in some other group of animals.

Recently I have been able to test the color of the luminescence resulting when luciferin and luciferase of two species of ostracods are "crossed." The luminescence of one form, *Cypridina hilgendorfii*, from Japan, is decidedly bluish. The luminescence of the other form, *Cypripina* (?), from Jamaica, B. W. I., is bluish yellow or yellowish. When dried specimens of the two ostracods are ground in separate mortars and water added to each, the difference in shade is unmistakable.

As a luminescence appears of a somewhat different shade depending on its brightness, it is necessary to prepare luciferin and luciferase of the same strength from each species. This was attempted by extracting 20 mgm. of dried ostracods of each species with 10 cc. distilled water and filtering. The luminescence disappears in a short time in these 0.2 per cent extracts.

Luciferin solutions of the two species were then prepared and the following mixtures made at a temperature of 22°C.

Japanese luciferase x Japanese luciferin – bluish light
Jamaican luciferase x Japanese luciferin –yellowish light

[3] The luciferase, an enzyme, is not used up.
[4] Granulolysis would perhaps be a better but somewhat awkward term.

Japanese luciferase x Jamaican luciferin – bluish light
Jamaican luciferase x Jamaican luciferin – yellowish light

It will be noted that the resulting luminescence presented the shade of that species supplying the luciferase. The light was slightly brighter and disappeared somewhat more quickly in all mixtures with Jamaican luciferase. Amberson (7) finds that with more concentrated luciferase the light is brighter and disappears more quickly. Consequently, I conclude that, although equal weight of dried material had been used in preparing the luciferase solutions, the Jamaican luciferase was really somewhat more concentrated.[5] Other experiments, however, using more concentrated Japanese luciferase, gave exactly similar results as far as the *color* of the light was concerned, although mixtures with the Japanese luciferase in this case were somewhat brighter and disappeared more quickly than those with Jamaican luciferase.

The luciferase solutions gave no luminescence when distilled water was added to them, or with chloroform, saponin or sodium glycocholate, so that cytolysis of photogenic cells or granules does not complicate the results. We may therefore conclude that in ostracods, as in the fire-fly, that form supplying the luciferase determines the color of the light, and that this is true when granulolysis of photogenic granules and differences in concentration of photogenic substances have been ruled out.

One might have predicted that the animal supplying the luciferin would determine the color of the light inasmuch as luciferin is the oxidizable substance. However, the color of the light appears to depend not so much upon what is oxidized as upon how it is oxidized. Luciferase is absolutely essential for luminescence. Luciferin oxidizes in absence of luciferase but without luminescence and I have found no oxidizing agent or enzyme to take the place of luciferase. Indeed, luciferin alone may be oxidized more quickly at high temperatures without luminescence than at low temperatures in presence of luciferase and with luminescence. It is obvious that rate of oxidation alone does not determine whether luminescence will appear. The presence of luciferase determines whether there will be luminescence. Provided luciferase is present, with a given amount of luciferin, the light will be brighter and will last a shorter time the greater the concentration of luciferase. The facts seem to indicate that light appears during a combination between luciferin and luciferase. Increased reaction velocity then gives a brighter light.

The effect of temperature is also to increase the intensity of the light of luminous animals to a limit (an optimum) above which the intensity diminishes. This is especially well seen in the luminous bacteria.[6] In

[5] Due to the fact that this species contains more luciferase or that it had been dried more recently that the Japanese *Cypridina* material.

[6] Quantitative results, not yet published, have been obtained by T. F. Morrison. The optimum is about 23°C. in bacteria.

Cypridina the light lasts a shorter time the higher the temperature, even above the temperature of optimum intensity. From the diminution in intensity above the optimum one would conclude that the reaction velocity decreased at very high temperatures, while from the rapid decay of luminescence at very high temperatures one should conclude that the reaction velocity was increased. The rapid decay at high temperatures is no doubt due to an effect of temperature on the spontaneous oxidation of luciferin which proceeds side by side with the luciferase oxidation of luciferin. Temperature seems to affect the action of luciferase apart from its influence on reaction velocity and the question arises as to an effect on the color of the light.

Dried Japanese *Cypridina* powder kept at various temperatures and moistened with water at the corresponding temperatures does exhibit color differences of luminescence. At extremes of temperature, 0° and 50°, (the light disappears somewhat below freezing and about 54°C.) the intensity is low but of about the same brightness and decidedly yellow at the high temperature. If we compare the light at 10° and 40° where it is quite bright, we find the shade distinctly more yellow at the higher temperature.[7] In fact the color of the Japanese *Cypridina* at 40° is as yellow as the Jamaican *Cypripina* at 20°, while the Japanese form at 20° is distinctly more blue than the Jamaican form at 20°. There is no doubt but that the Japanese ostracod (*Cypridina*) light shifts toward the longer wave-lengths the higher the temperature. This also occurs in the Jamaican ostracod (*Cypripina*) but not so clearly defined.

The same is true of the fire-fly. A species of *Photinus* with a yellow light obtained at the Nela Research Laboratory, Cleveland, Ohio, becomes more greenish around 0°[8] and more orange around 45°C. although the intensity is about the same. There is a shift toward the longer wave-lengths with increase in temperature just as in *Cypridina*.

Whether we can conclude that this shift in wave-length is connected with reaction velocity is doubtful. One is inclined to look for effects of temperature upon luciferase, perhaps its aggregation state, since we know that at still higher temperatures coagulation occurs. As aggregation state is a rather vague term, perhaps the most one can conclude is that the difference between the luciferases of the Japanese *Cypridina* and the Jamaican *Cypripina* is similar to the difference brought about by temperature change in the luciferase of the same species of luminous animal.

[7] In my book (*The Nature of Animal Light*, 1920, pp. 156 and 160) the statement is made that *Cypridina* light is more yellow at high temperatures and also at 0°C. There is no doubt of the yellow color of high temperature luminescence but for low temperature *with equal brightness* I cannot confirm the yellow appearance. The color is more blue at the low temperatures.

[8] McDermott (8) reports the light of the fire-fly to become more reddish just before disappearing in liquid air.

SUMMARY

When luciferase and luciferin of two species of ostracods having different colored luminescences are intermixed, the resulting luminescence color is determined by the animal supplying the luciferase.

In the fire-fly and in ostracods, increase in temperature shifts the color of the luminescence toward the longer wave-lengths.

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THE COMPOUND NATURE OF THE ACTION CURRENT OF NERVE AS DISCLOSED BY THE CATHODE RAY OSCILLOGRAPH

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WITH THE COLLABORATION, IN SOME OF THE EXPERIMENTS, OF

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Records of the monophasic action current in nerve obtained by means of an inertialess method consisting of a three-stage amplifier working into a cathode ray oscillograph often exhibit waves during the falling phase. Such action currents were pictured and briefly discussed in our first publication on this subject (1). A more thorough study of action currents with a view primarily toward ascertaining the nature of these waves and their significance formed the original purpose of the present investigation.[1] As the problem developed it became necessary to investigate the changes in the form of the action current that occur in association with its propagation. The results thus disclosed together with their experimental analysis form the subject of this paper.

METHODS. The methods employed in general have differed only in detail from those described in our previous publication. The same oscillograph tube lasted well along into the present research. The two tubes that have been employed since in some respects were superior to the first. The newer tubes are now provided with larger deflecting plates which considerably increase their sensitivity. In the case of the second tube, for example, 11 volts applied to the deflecting plates deflected the spot of light made by the rays on the fluorescent screen a distance of 1 cm., whereas, to produce the same deflection it was necessary, in the case of the first tube, to impress 23 volts upon the deflecting plates. The increase in the size of the plates in addition has the effect of making the tube practically equally sensitive in all positions of the spot within the utilized range of the screen. Consequently it no longer is necessary to correct records for the decrease in vertical sensitivity as the deflection

[1] Some of the results have been reported before the American Physiological Society (2).

increases horizontally from left to right. It so happens in the case of our third tube, however, that the vertical deflections are not at right angles to the horizontal deflections, probably because of defective position of the plates. It consequently has been necessary to make an angular correction for all points not on the horizontal axis. To obtain the positions of such points in a system of rectangular coördinates the records have been measured as though their X and Y axes were at right angles to each other and then the vertical measurements have been multiplied by the tangent of the angular deformation of the tube's coördinate system, namely, 0.0646, to obtain the value to be subtracted from the abscissa. The increased sensitivity of the oscillograph renders unnecessary the extra length of the linear portion of the amplifier characteristic produced by using in the third panel a 300 volt plate battery with the addition of a grid battery as previously described (1). We are therefore using a 150 volt plate battery and omit the grid battery. We have also found that one plate and one filament battery, supplying both the first and second panels, is satisfactory.

We have come more and more to make contact prints of the standing waves on the face of the tube rather than trust to tracings. The contact prints, owing to radiation are not quite so sharp in outline as tracings, but they have the advantage of being true and objective. If a lead pencil line is drawn through the darkest part of the film, as viewed in strong transmitted light, measurements can be made with the greatest of accuracy through a glass coördinate scale ruled in millimeters. The traced or photographed standing waves thus obtained, after the angular correction where necessary, are on rectangular semi-logarithmic coördinates. They can be transferred to linear coördinates as described in our previous publication. The slight distortion of the records produced by the curvature of the fluorescent screen has not been corrected; as an effort has been made to keep important points on or close to the great meridians, the error from this source usually is quite negligible.

In many of the experiments more recently performed, in order to shorten the duration of the induction shock, a non-reacting resistance of 100,000 ohms has been inserted in series in the secondary circuit of the inductorium used for nerve stimulation. For the same reason in some of the experiments the core has been removed from the secondary coil. Rather recently it was discovered by directly observing the "shocks" on the fluorescent screen, that the simple induced break shock is converted by the high secondary resistance into an oscillating current. As we have not been directly concerned with this phenomenon, which was originally observed by Helmholz (3), it has been investigated only casually. The oscillations were some five or six in number, rapidly declining in amplitude, and of a period roughly of 0.17σ ($1\sigma = 0.001$ second). It

seems fair to conclude on account of their very rapid decline in amplitude that only the first of these oscillations ever reached the threshold of the nerve's irritability. However this may be, it may be stated once and for all that in so far as concerns the subjects treated in this paper the monophasic and the oscillating induction shocks have yielded absolutely identical results.

EFFECT OF THE DISTANCE OF CONDUCTION ON THE CONFIGURATION OF THE ACTION CURRENT. Light upon the fundamental nature of the waves which the cathode ray oscillograph has disclosed in the amplified action current was first obtained in experiments designed to ascertain whether altering the distance the action current is propagated along the nerve affects the relative positions of the waves. In these experiments the nerve is mounted in a moist chamber and kept at a constant temperature. The leads from the killed end and intact side of the nerve through the amplifier into the cathode ray tube consist of non-polarizable electrodes of the Zn — $ZnSO_4$ — NaCl type. The stimulus is delivered through pairs of platinum electrodes of which several, 3 to 5, range along the nerve at measured distances from the proximal lead into the oscillograph. By means of double-pole, double-throw switches situated outside the moist chamber any desired pair of electrodes can be connected with the inductorium. In a few experiments but a single pair of stimulating electrodes was fixed to the central end of the nerve and a number, 3 to 4, of non-polarizable side leads at measured distances along the length of the nerve were so connected with a pole selector outside the moist chamber that any one of them could be put in circuit with the dead end of the nerve through the recording apparatus. It should be stated, however, that the latter arrangement has the disadvantage that the number of fibers in contact with the side leads diminishes with the distance of the leads from the stimulating electrodes; and that the resistance in the oscillograph circuit differs with each lead. The contacts between the leads, as will be explained later, also affect the form of the action current. Excepting, therefore, as a means of determining propagation rate, this method has proven less valuable than the one first described above, in which the nerve fibers concerned in producing the recorded action current and the resistance remain the same. As is customary in experiments of this type, the proximal stimulating electrode (with respect to the leads into the oscillograph) was cathode on the break. The effect of reversing the direction of the stimulating current was observed from time to time in order to assure ourselves that we were dealing with action currents. We will concern ourselves here with descending conduction mainly. It may be added, however, that the waves have been seen in the ascending action current also.[2]

[2] In collaboration with G. H. Bishop.

THE ACTION CURRENT IN THE PHRENIC NERVE. These experiments on the form of the action current at different points in its passage along the nerve have brought out the fact that it undergoes a number of changes in its progress from the point to which the induction shock is applied. These changes include not only the separation out of waves, which was the phenomenon that was originally singled out for study, but other changes as well. As all of these alterations are taking place simultaneously and complicating one another, it seems best in the interest of clearness to describe first the progressive changes as they present themselves in a nerve, such as the phrenic of warm-blooded animals, in which waves do not separate out at any conducting distance. It then becomes a simple matter to ascertain, in the case of action currents composed of waves, the part they play in the observed change in the form of the action current with its progression.

Contact prints of an illustrative series of action currents in the phrenic nerve of the dog are reproduced in figure 1a (see also 1b). It will be noted in the first place that there are no catacrotic waves on these action currents at any conducting distance. This has been the case without exception in the phrenic nerve. Analysis shows further (see table 1, 5, 16, '23) that with the propagation of the action current (from 1.35 cm. to 10.9 cm.) there occur *a*, a progressive increase in the time to maximum (from 0.42 to 0.66σ), and *b*, in the width of the crest, *c*, a progressive decrease in amplitude (from 39.6 [18][3] to 26.5 mm.) and *d*, a progressive increase in duration of the quicker part of the deflection (1.0 to 1.59σ).[4] Table 1 contains the analysis of a similar experiment (1, 25 '23) in which the series were thrice repeated in succession. The fact illustrated by these series that the same sequence of changes recurs upon repetition excludes fatigue as a factor in the production of the alteration in the form

[3] The unmodified deflections were higher than the screen, and the potential was reduced to the desired height by placing the required number of 47,500$^{\omega}$ nonreactive resistances in parallel with the nerve. When the resistance of the nerve is ascertained the fractional reduction of the nerve potential by these resistances can be calculated. Here the resistance of the nerve unfortunately is not known. If the precarious assumption be made that the resistance of this nerve between the leads was the same as in a similar case in which the measured resistance was 21,500$^{\omega}$, then multiplying the amplitude of the derived action current of part A, figure 1 a, by the factor 2.2 would give the amplitude for comparison with the observed amplitudes in B, C and D. The value expressing the amplitude of A in the text and in the table has thus been derived from the bracketed value.

[4] The termination of the action current is very gradual and usually, with sufficient amplification, is beyond the end of the record made with the rapid deflection rate of the fluorescent spot needed for present purposes. It is usually possible, however, to recognize a point on the record where the descending limb of the action current becomes practically parallel to the base line, and the term "end of the action current," is used throughout this paper to denote that point.

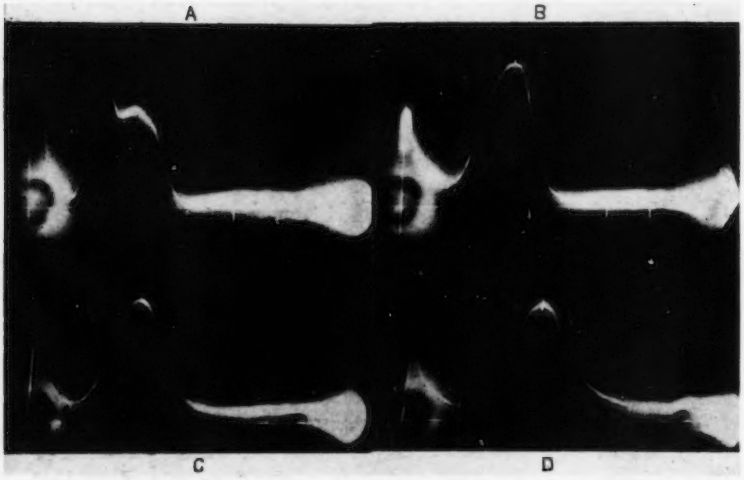

Fig. 1a (expt. 5, 16, '23–1). Contact prints of the action current in the phrenic nerve of the dog. Conducting distances in millimeters: A = 13.5, B = 42.5, C = 78.5, D = 109.0. Temp. = 37.5°C.; X = 7.6 cm.; 1 mf.; 2,000$^{\omega}$; potential fractioned in A by 9,500$^{\omega}$; in B, C and D by 47,000$^{\omega}$. The marks extending below the base line indicate successive σ. $\times \frac{2}{3}$.

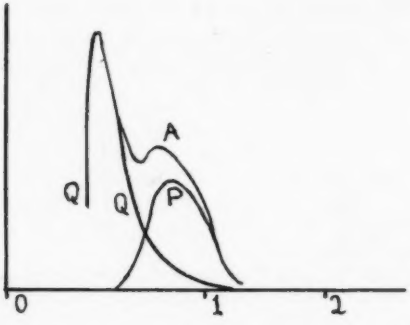

Fig. 1b. Derivation of the action current from *A*, fig. 1a. *A* = *A* of figure 1a. *Q* = escape of stimulating current determined by method a, page 656. *P* is the action current derived by subtracting *Q* from *A*. The marks extending below the base line indicate successive σ. Natural size.

of the action current as it moves along the nerve. Discussion of the significance of these phenomena may be postponed until certain other data have been presented and we may now turn to a consideration of a nerve, the bull frog's sciatic, best suited, on account of its length, to a study of an action current composed of waves.

THE COMPOUND ACTION CURRENT IN THE SCIATIC NERVE OF THE BULL FROG (*Rana Catesbiana*). Figure 2a consists of direct contact prints of the action current in the sciatic nerve of the bull frog (expt. 5, 23, '23-2)

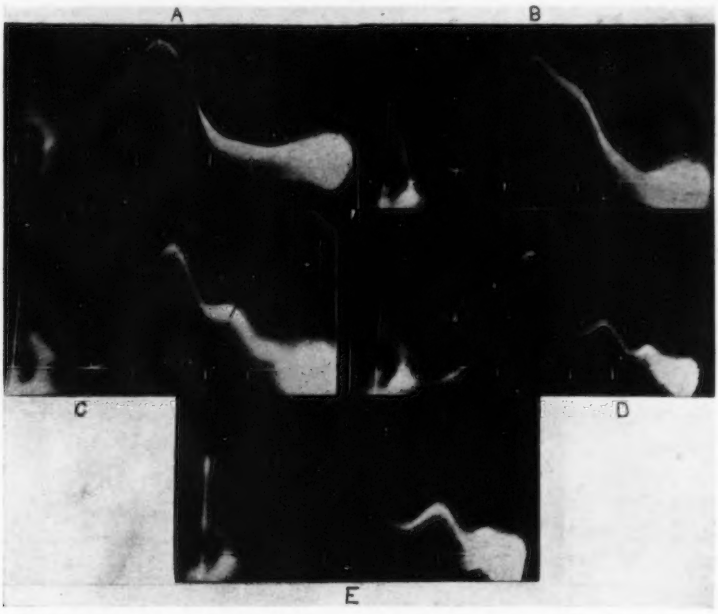

Fig. 2a (expt. 5, 23, '23-2). Contact prints of the action current in the sciatic nerve of the bull frog. Conducting distances in millimeters: A = 12.0, B = 31.0, C = 46.5, D = 62.5, E = 82.0. Temp. = 26°C.; X = 7.6 cm.; 1 mf.; 2,000$^{\omega}$; input fractioned in A by 5,938; in B, C, D and E by 23,750$^{\omega}$. Distance between the lines extending below the base line indicate successive σ. × $\frac{33}{35}$.

obtained at five separations of the cathode of the stimulating electrodes from the proximal one of the oscillograph leads, namely, 12, 31, 46.5, 62.5 and 82 mm. in parts A, B, C, D and E respectively of the figure. Figure 2b shows the same records transposed from the original eighty-seven degree, eighteen minute and semi-logarithmic system of coördinates of the oscillograph to ninety degrees, linear coördinates. The horizontal axis of this system gives the time in sigma; the vertical axis gives the height of the action current in millimeters as measured from the base

line of each, and these base lines are spaced by distances from the main horizontal axis that are proportional to the respective conducting distances. Finally, the beginning of each action current has been set on zero time. Two additional sets of similarly replotted tracings are shown as figures 3 and 4. In the latter the highest crest of each of the action

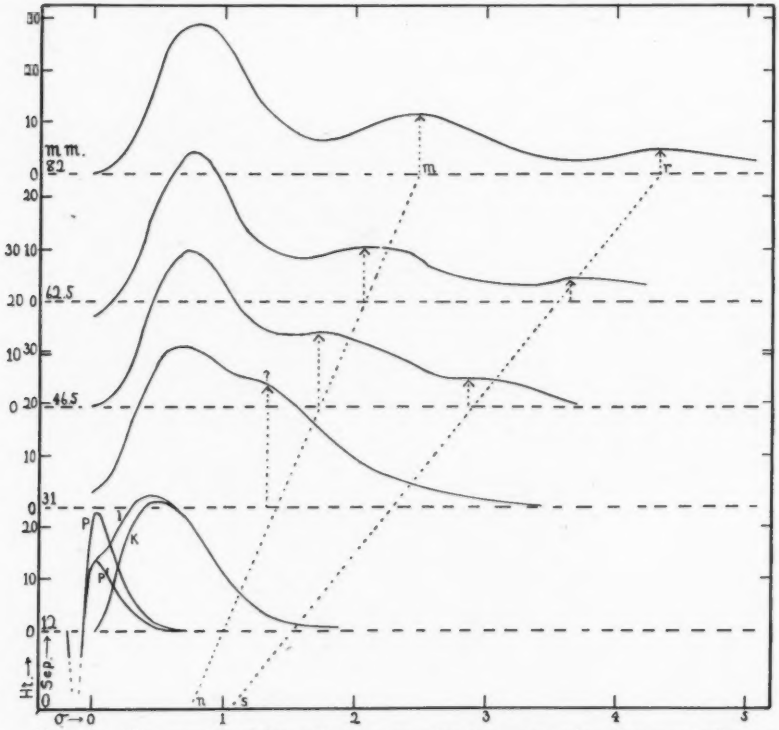

Fig. 2b. The records of figure 2a transposed onto rectangular, linear coördinates. Abscissae give the time in sigma; ordinates, *Sep.*, the conducted distance, with zero distance at the X axis, and the amplitudes of the action currents in millimeters, *Ht*, as measured from the base line of each record. The dotted lines *m-n* and *r-s* are seen to nearly join the projections of the crests of the beta and gamma waves respectively upon their respective base lines.

currents has been set on the abscissa of 0.8σ by arbitrarily altering the times to maximum, and certain additions have been made to which reference will be made later on. The lowermost set of figure 2b includes a construction necessary for the derivation of the action current, *k*, from the merged action current and escape of the stimulating current, *l*. The form of the escape, *p*, was got by method c, page 656. The recorded

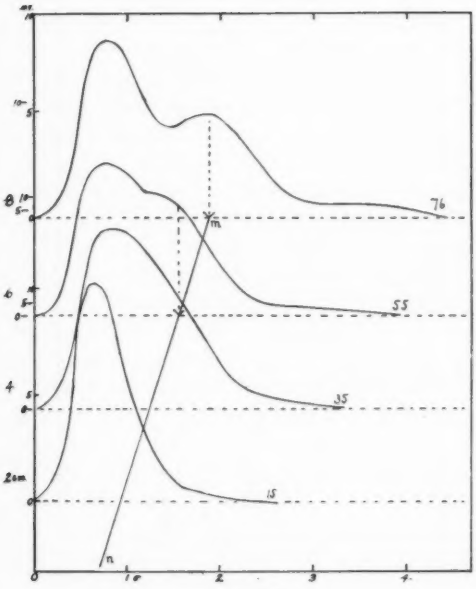

Fig. 3

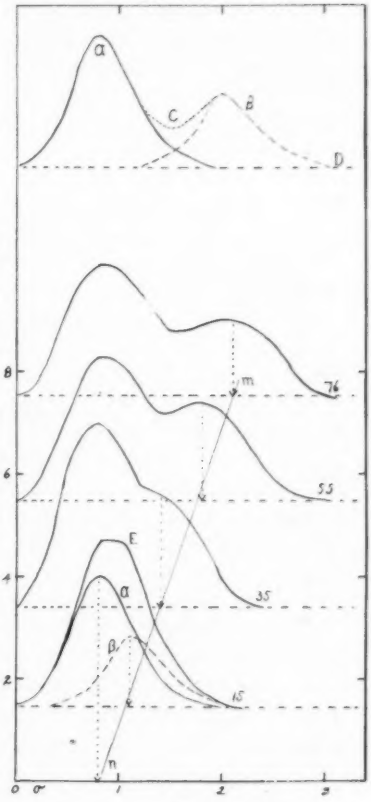

Fig. 4

Fig. 3 (expt. 12, 15, '22). Tracings of the action current in the sciatic nerve of the bull frog transferred to linear coördinates. Temp. = 22.3°C. In the originals X = 7.98 cm.; 1 mf.; 5,000ω; full input. Abscissae give the time in sigma; the ordinates the conducting distances in cemtimeters, with zero at the X axis, and the amplitudes of the action currents in millivolts with zero at the base line of each of the action currents; *m-n* as in figure 2b.

Fig. 4 (expt. 12, 14, '22). Tracings of the action current in the sciatic nerve of the bull frog, transferred to linear coördinates, after arbitrarily making the time to maximum of the main crests alike and equal to that of the slowest. The constructions in the lowermost and uppermost groups are described in the text; *E* is the action current that belongs to the series. Otherwise as in figure 3.

escape, p, however, rises higher than the current of the combined escape and action current, l. In order to make subtraction possible the record of the escape has been reduced vertically, p', without altering it horizontally, so as to make it fit into the combination record, l.

Inspection of these figures shows that at the longer conducting distances the action current is composed of three waves, though the third in some instances is not very distinct. These waves may be designated alpha, beta and gamma from before backwards in the action current. Occasionally a fourth and still smaller, delta, wave is seen. These figures give the very definite impression that the shift in the relative positions of the waves that occurs with the change in the conducting distance, is due to differences in the rates with which the waves move along the nerve. When this conception first suggested itself to us diagrams similar to the ones reproduced in figure 5 were drawn in order to obtain a basis for the analysis of the records. The values employed in the construction of this figure have not been selected arbitrarily; as a matter of fact, they have in the main been derived from the experiment illustrated by figure 4, as will become evident later. Figure 5 puts in graphic form the effect of the summation of two waves, one large, α, the other small, β, both of approximately the same duration, starting together, but traveling at different though uniform rates, alpha at 42 M.p.s. and beta at 25 M.p.s. The individual waves in each case are drawn in light lines; their sum, where summation occurs, in heavy lines. The cases selected depict lags of beta behind alpha of 0, 0.4, 0.6, 0.8, 1.0 and 1.5σ respectively as indicated in the figure.

The distance alpha must travel for these lags to develop may be calculated as follows: Let $V\alpha$ and $V\beta$ represent the propagation rates of alpha and beta. The time required for alpha to travel the distance, d, is $\frac{d}{V\alpha}$, and for beta to travel the same distance, $\frac{d}{V\beta}$ The difference between these two expressions then gives the lag, l_β, of the slower wave, or

$$l_\beta = \frac{d}{V_\beta} - \frac{d}{V_\alpha} \qquad (1); \text{ whence}$$

$$d = \frac{l_\beta V_\beta}{1 - \frac{V_\beta}{V_\alpha}} \qquad (2)$$

It is thus found that the distances alpha must travel to develop the lags depicted in the figure are for 0.4σ (b) 24.8 mm., for 0.6σ (c) 37.2 mm., for 0.8σ (d) 49.6 mm., for 1.0σ (e) 62 mm., and for 1.5σ (f) 93 mm. In the figure, each pair of alpha and beta waves and their sum are plotted on that ordinate as a base which corresponds to the distance in centimeters alpha has traveled (indicated on the ordinates); the time in σ it is indicated on the base line.

It will be noted that the changes occurring in these summed waves as the conducted distance increases very closely resemble in form those exhibited by the action current in the bull frog's sciatic nerve. There occur not only an almost identical evolution of waves, but also a diminution in amplitude and an increase in duration. There are, however, certain differences that remain to be explained. In the diagram (fig. 5) the main crest at first broadens and then narrows again to its original width as the crest of beta emerges. The crest of the action current (figs. 2, a and b, 3, 4) also at first broadens and then narrows again somewhat as the crest of beta appears; but its crest broadens again subsequently with the further progression of the action current. Again, whereas in the diagram (fig. 5) the time to maximum of the summed waves at first increases and then decreases to its original value as beta emerges, in the action currents the time to maximum increases constantly, though somewhat less rapidly at about the time the crest of beta emerges. And finally the amplitude of the summed waves in the diagram diminishes only as long as beta is coming out from under alpha; whereas in the action current the amplitude continues to diminish even beyond what seems to correspond with the above-mentioned stage of separation. These differences between the diagram and the actual action current can

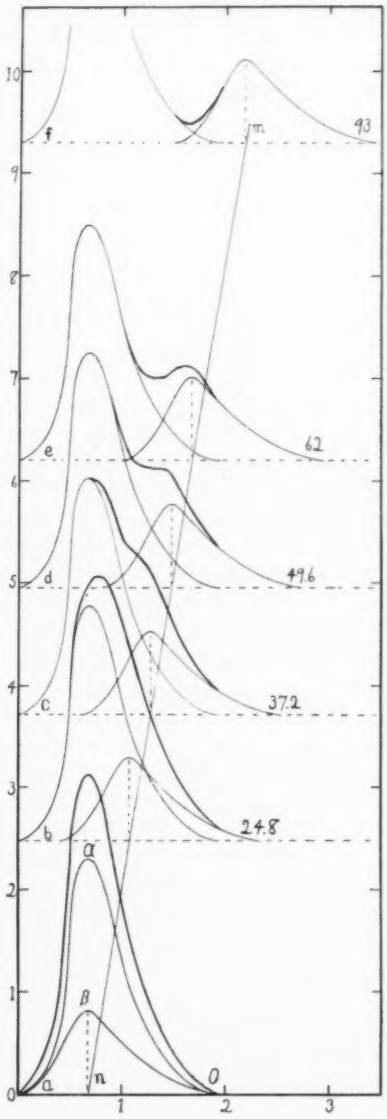

Fig. 5. Diagram showing the summation of two waves, α and β, starting together but traveling at different rates. Ordinates give the propagated distance in centimeters; abscissae the time in σ. Description in text.

be readily reconciled, though, if it be assumed that in the frog's sciatic two types of progressive changes are occurring simultaneously, namely, *a*, a dissociation of the action current into discrete waves, and *b*, alterations in the form of each of the discrete waves similar in kind to those exhibited by the action current of the phrenic nerve with its progression.

Though the diagram was constructed to elucidate and to furnish a basis for the analysis of the waves in the frog's action current it has served also to suggest a possible explanation of the progressive change in the form of the phrenic action current and of each of the constituent waves of the frog's action current. It suggests that these changes might have as their basis a process essentially the same in kind as that which brings about the changes in the waved form of the action current. A progressive diminution in amplitude, widening of crest, increase in time to maximum, increase in duration, etc., which the gross waves display would occur if they were composed of a large number of waves starting together but traveling at slightly different rates. Other possible factors leading to these changes are *a*, extinction of action currents in a certain number of fibers due to injury or the abnormal conditions to which the nerve is exposed, and *b*, so-called conduction with a decrement. Further work is being done in an effort to obtain more light on this phenomenon.

The diagram (fig. 5) shows that it is not a simple matter to locate in the summed waves the significant features of the component waves. Thus neither the notch between the summed waves, nor the highest point of either the alpha or the beta elevations on the summed waves necessarily mark the positions of the beginnings or of the crests of the component waves. It is clear, however, that with the shifting relation of the waves to each other a crest made by beta in the summed curves, shifts less from the actual position of the crest of beta alone than does the notch shift with respect to the beginning of beta. It is obvious, therefore, that unless definite information is available with regard to the actual form of the waves composing an action current, and usually it is not, the location of significant corresponding points on the action currents obtained at different propagation distances is in part a matter of judgment. But in any event a distinct crest on the action current must be regarded as a much better, though not an exact, index to the position of the crest of the wave producing it than is a notch to the position of the start of the wave, excepting where the waves have separated to such an extent that the notch descends quite to the base line.

The diagram (fig. 5) is so constructed that corresponding points on the several curves when projected upon their respective base lines fall upon a straight line. This is illustrated by the line *m-n* which joins the crests of beta thus projected. If, therefore, the action currents were composed, as are the compounded waves of the diagram, of waves each

constant in form, starting simultaneously and traveling each at a uniform rate, the projection points of beta, for example, would fall on a straight line which would intersect the axis of the abscissae at a distance from zero equal to the time to maximum of beta. It has been seen, however, that the component waves of the action current change their form and time to maximum as they progress. Nevertheless the projected crests of the beta waves of the action current, as may be seen in figures 2b and 3, actually do fall fairly well upon such a straight line. This line, however, intersects the main horizontal axis somewhat further from zero than the time to maximum of the action current obtained at the shortest conducting distance, and roughly at about the time to maximum of the alpha (and of the beta) wave of the farthest conducted action current. The same is true also of the line joining the crests of gamma in figure 2b, though here the experimental error naturally is considerably larger.

In order to obviate as far as possible the complication caused by the progressive change in the form of the constituent waves of the action current with their progression, in figure 4, as has been said, the crests of the alpha processes have been placed upon the same abscissa by arbitrarily making the times to maximum all equal in duration to the longest of them. Here it is seen that the projection points of the beta waves fall fairly well on a straight line which is so inclined as to intersect the main horizontal axis at the intersection point of the abscissa of the main crests.

These observations indicate quite definitely that the action current in the bull frog's sciatic nerve is made up of three (rarely four) independent waves of potential starting simultaneously, or almost simultaneously, from the point stimulated and traveling at different, though uniform, rates along the nerve, each wave in turn changing its form slightly as it progresses. It may be stated now that every observation we have made is consistent with this conclusion. Furthermore, every observation we have made indicates that each wave represents a discrete action current started by the one stimulus, but traveling in different groups of nerve fibers. Thus each wave or better, perhaps, each group of fibers, it will be shown, has its own conduction rate, its own threshold of stimulation, and its own refractory period.

Cause of summation. Before presenting this evidence, however, it might be well to consider why the recorded potential is higher when the waves overlap and give the appearance of summation. Our recording mechanism tends to act as a potentiometer. Nevertheless, owing to the peculiar structure and function of the source of potential, the nerve, effects can develop which simulate summation of potential. To make this clear we may regard the nerve as being composed in part of fibers in which the changes develop that determine the potential differences between the leads, and in part of inert substance, both being relatively poor conductors of electricity.

Considering first the case of a just maximal stimulus which causes all of the fibers under the leads to become active, it is obvious that the full potential difference developing in the individual fibers will not be indicated because the inert substance of the nerve acts as a shunt to the active substance. If the strength of the stimulus be appropriately reduced not even all of the reactive tissue will develop potential, so that now the potential in the active fibers will be shunted not only by inert tissue, but by the inactive nerve fibers as well. And finally, the full potential developed, excluding the shunting effect by the inert substance, would only manifest itself when the potential in all the fibers is the same. In case some of the fibers have a lower actual potential than others, the recorded potential would be somewhat less than the higher one due to the shunting effect of the fibers of lower potential. Therefore when,

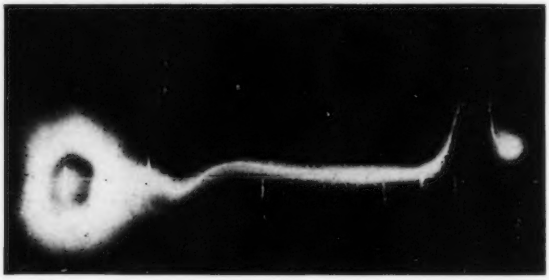

Fig. 6 (expt. 5, 23, '23–1). Contact print illustrating the method of determining the propagation rate of the action current. The conducting distance was 82 mm. The first downward deflection is the escape of the shock, the large upward deflection the action current. Temp. = 26°C.; X = 7.6 cm.; 1 mf.; 2,000$^{\omega}$. The longer lines extending below the base line indicate successive σ. Natural size.

due to differences in conduction rates, waves get out of phase the situation is equivalent to fibers of a higher potential shunted by fibers of a lower, and the recorded potential falls. In other words, the potential difference between the leads is determined both by the relative number of nerve fibers active and by any inequalities of potential of the component fibers at any given instant.

Propagation rates of the waves. It has been stated that each of the waves composing the action current has its own propagation rate. These rates may be approximately ascertained as follows. The rate of alpha ($V\alpha$) first is directly determined by calculation based upon a, the time elapsing between the start of the so-called escape of the induction shock and the start of the ensuing action current as measured in oscillograph records (see fig. 6), and upon b, the conducting distance, d, between the

stimulating electrode (cathode) and the proximal lead from the nerve. Then by a rearrangement of formula (1) we have

$$V_\beta = \frac{d}{l_\beta + \frac{d}{V_\alpha}}, \qquad (3)$$

$$V_\gamma = \frac{d}{l_\gamma + \frac{d}{V_\alpha}}, \text{ etc.} \qquad (4)$$

The lag (l_β, l_γ, etc.) of the beta and gamma waves is the time elapsing between the beginning of these waves and the beginning of alpha after propagation over a given distance. It has been seen, however, that the positions of the starts of the slower waves can not be ascertained with even a reasonable degree of accuracy. We, therefore, have taken the times elapsing between the crests of alpha and of beta, gamma, etc., as the lags between these waves. This is justifiable because, as has been pointed out, these crests at fairly long conducting distances are not materially displaced by summation, and because, as will be seen later, the times to maximum of all of the waves are essentially alike.

Our method of determining the propagation rate of the foot of the action current is by no means free of error. Some of the error is inherent in the principle, some in the nature of the preparation. In measuring as we do from the escape of the stimulating current to the beginning of the action current, no account is taken of a possible interval between the start of the shock and the start of the ensuing action current, nor of the spread of the stimulus beyond the cathode. We have obtained a certain amount of information with regard to the influence of these factors, but not enough to justify any definite statements at this time. It may be stated, however, that in our experiments the shocks were very brief. And it will be shown that if the propagated disturbance started by these induction shocks does not start at once it certainly does within 0.06σ. With strong shocks there is some evidence indicating that the stimulus may spread as far from the cathode as 9 to 10 mm. Then there is the possibility of a considerable error due to the difficulty in recognizing exactly the position of the start of the action current because of its gradualness. Further experiments are being done in an effort to ascertain more exactly the significance of these factors. By employing only long preparations, in which the effects of the initial disturbing influences are minimal, our method of determining the propagation rate becomes quite sufficient for present purposes. A number of our observations on conduction rate have been collected in table 1, where it will be seen that the conduction rate of the foot of the action current in the case of a given nerve of any given species lies within certain well-defined limits. This,

TABLE 1

EXPERIMENT	PREPARATION	TEMPERATURE	CONDUCTED DISTANCE	CONDUCTION RATE M.p.s.		HEIGHT OF MAIN WAVE	SHUNT	TIME TO MAXIMUM	DURATION
				α	$\beta\ \gamma\ \delta$				
			mm.			*mm.*	ω	σ	σ
11/10/22	Bull frog sciati	22.8	79.0	42.6	–	–	–	–	–
11/11/22	Bull frog sciatic	–	72.0	41.1	–	–	–	0.70	–
11/17/22	Bull frog sciatic	–	70.0	44.3	–	–	–	0.73	–
12/ 2/22	Bull frog sciatic	24.4	30.0	34.8	–	32.5	0	0.62	–
			50.0	48.2	–	27.0	0	0.65	–
			70.0	47.3	β28.7 γ19.0 δ13.6	19.0	0	0.69	–
12/14/22	Bull frog sciatic	–	15.0	–		31.0	0	0.44(?)	1.81
			35.0	42.7		28.5	0	0.46	2.04
			55.0	41.0		28.0	0	0.74	3.47
			76.0	41.8	β25.1	26.0	0	0.83	3.14
12/15/22	Bull frog sciatic	22.3	15.0	–	–	48.0	23,700	0.55	2.50
			35.0	35.2	–	38.5	23,700	0.68	3.26
			55.0	40.9	–	33.0	23 700	0.76	3.95
			76.0	43.8	β27.0 γ16.2	36.3	23,700	0.77	5.45
3/21/23 (1)	Bull frog sciatic	21.0	17.0	–	–	33.0	11,875	0.53	1.76
			45.0	–	–	24.0	11,875	0.87(?)	3.11
			73.0	41.3	β25.6	22.5	11,875	0.78	3.77

3/21/23 (2)	Bull frog sciatic	21.0	17.0	–	–	42.0–		15,833	0.45+	–
			45.0	–	–	28.5		15,833	0.67	–
			73.0	41.3	β22.1(?)	27.0		15,833	0.84	–
5/23/23 (1)	Bull frog sciatic	26.0	12.0	41.5	–	[18.0]*	73.1	5,938	0.41–	–
			31.0	40.1	–	[31.0]	36.5	15,833	0.66	–
			46.5	38.2	–	[38.0]		23,750	0.74	–
			62.5	40.0	–	[35.0]		23,750	0.79	–
			82.0	44.2	β24.2	[34.0]		23,750	0.80	–
5/23/23 (2)	Bull frog sciatic	26.0(?)	12.0	–	–	[24.0]	96.0	5,938	0.46	E = 1.88
			31.0	–	–	[30.0]		23,750	0.70	E = 2.91+
			46.5	–	–	[29.8]		23,750	0.76	C = 2.87
			62.5	–	–	[28.2]		23,750	0.75	C = 3.65
			82.0	41.6	β22.4 γ14.9	[29.0		23,750	0.80	C = 4.34
6/ 2/23	Bull frog sciatic	–	82.0	38.2	β25.9 26.3	–		–	–	–
11/18/22	Green frog	24.5	40.0	35.5	β18.4(?) γ12.2(?)	–		–	0.54	–
11/20/22	Green frog	21.6	45.0	29.8	β12.2(?) γ 7.8(?)	–		–	0.51	–
11/22/22	Green frog	23.3	10.0	–	–	41.0		0	0.52	2.00(?)
			20.0	–	–	36.0		0	0.54	2.22(?)
			30.0	–	–	29.0		0	0.57	2.84(?)
			40.0	33.3	β17.0 γ 9.4(?)	28.0		0	0.59	3.21(?)

TABLE 1—*Concluded*

EXPERIMENT	PREPARATION	TEMPERATURE	CONDUCTED DISTANCE	CONDUCTION RATE M.p.s.		HEIGHT OF MAIN WAVE	SHUNT	TIME TO MAXIMUM	DURATION
				α	$\beta\ \gamma\ \delta$				
			mm.			*mm.*	ω	σ	σ
5/18/23	Green frog	—	5.0	—	—	[21.0] 34.9	9,500	0.43	1.40
			15.0	—	—	[37.0]	15,833	0.51	1.95
			24.5	27.1	—	[32.0]	15,833	0.51	2.08
11/29/22	Dog phrenic	38.2	102.0	50.0	—	—	—	0.51	1.79
12/16/22	Dog left saphenous	38.5	37.0	53.2	—	24.8	0	0.48	1.67
			77.0	71.5	β51.7	17.5	0	0.50	1.77
	Right saphenous	38.0	37.0	—	—	17.5	0	0.30(?)	1.61
			64.0	64.8	—	15.0	0	0.36	1.93
			95.0	83.3	β57.8	12.8	0	0.46	2.05
	Left tibial	38.0	30.0	—	—	23.5†	0	0.26	1.16
			55.0	80.4	—	33.0	0	0.43	1.38
	Right tibial	38.0	36.0	72.5	—	27.5	23,750	0.38	0.90
			82.0	87.6	—	30.5	(?)	0.54	2.14
	Left vagus	38.3	32.0	41.7	—	6.0	0	0.52	1.42
			87.0	53.6	—	4.0	0	0.74	2.57
	Right vagus	38.2	29.0	54.2	—	4.2	0	0.44	1.45
			80.0	58.7	—	4.0	0	0.77	2.67
	Left phrenic	38.7	110.0	52.9	—	37.5	0	0.69	2.48

1/25/23	Dog phrenic	38.6	25.0	39.7(?)	—	[31.5]	45.7	47,500	0.35	0.59‡
			59.0	46.0(?)	—	33.0		0	0.49	0.61‡
			87.3	40.2(?)	—	25.0		0	0.51	0.72‡
			25.0	—	—	[31.5]	45.7	47,500	0.39	0.51‡
			59.0	—	—	32.0		0	0.49	0.61‡
			87.3	—	—	24.0		0	0.48	0.71‡
			25.0	—	—	[29.0]	42.2	47,500	0.40	0.54‡
			59.0	—	—	30.0		0	0.49	0.64‡
			87.3	—	—	21.0		0	0.51	0.69‡
5/16/23	Dog phrenic	—	13.5	—	—	[18.0]	39.6	9,500	0.42±	1.00(?)
			42.5	—	—	33.0		47,500	0.47	1.16
			78.5	—	—	28.5		47,500	0.52	1.28
			109.0	73.5	—	26.5		47,500	0.66	1.59

* The bracketed figure gives the measured height, the other the height when allowance is made for the shunt.
† Deformed by "escape."
‡ End too gradual for measurement. These values give the width of the action current half way between base and crest.

however, is a matter that does not concern us now. The propagation rates of the waves may be illustrated by referring specificially to the rates determined in the experiments that form the basis of figures 2, 3 and 4. For figure 2 (expt. 5, 23, '23) the propagation rates of alpha, beta and gamma are 41.6, 22.4, and 14.9 meters per second respectively (26°C.); for figure 3 (expt. 12, 15, '22) 43.8, 27.0 and 16.2 M.p.s. for alpha, beta and gamma respectively (22.3°C.); and for figure 4 (expt. 12, 14, '22) 41.8 and 25.1 M.p.s. respectively for alpha and beta. These results are quite typical of the rates in the bull frog's sciatic nerve at these temperatures,[5] as may be seen in table 1. In only one nerve (expt. 12, 2, '22) has there been a delta wave of sufficient distinctness to justify calculation of its propagation rate. In this case the propagation rates of alpha, beta, gamma and delta (24.4°C.) were 47.3, 28.7, 19.0 and 13.6 M.p.s. It becomes clear, therefore, that the component waves of the action current have different propagation rates.

Stimulation thresholds of the constituent waves. In our first publication it was pointed out that waves may appear in action currents started by submaximal stimuli. By submaximal stimuli we then understood stimuli eliciting action currents of less than maximal height. It was also pointed out there that the waves are more definite when the strength of the stimulus is increased; that these variations in the prominence of the waves occur within a comparatively narrow range of stimulation strength; and that the duration of the disturbance is not much increased by varying the strength of the stimulus. While further experience with this phase of the subject has confirmed the above observations, it also has indicated the need of certain modifications in their interpretation.

When the conditions are such that the waves are prominent and well separated out, decreasing the strength of a supramaximal stimulus usually has the effect of causing the waves to diminish in amplitude and eventually to disappear in succession from the end toward the beginning of the action current. Thus at a certain stage in the weakening of the stimulus gamma, if present, first diminishes and disappears from view; then, shortly beta likewise diminishes and disappears, while alpha may yet have diminished but little, if any, in height. Eventually alpha begins to diminish and also disappears. At shorter conducting distances, however, where the waves, though evident, are not well separated out, diminishing the strength of the stimulus still causes them to disappear in succession; but now the alpha crest at the same time undergoes an apparent diminution in height due, as will be seen, to what in effect amounts to the subtraction from it of the gamma and beta waves. These were

[5] Unfortunately the temperature was not always read. All of the observations on cold-blooded nerve were, however, made at room temperature which usually lay between 22° and 24°C.

the circumstances that led us to make the statement quoted above that waves may be present when the stimulus is submaximal, but may be made more prominent by increasing the strength of the stimulus. Since we have come to recognize, and have learned to allow for, the compound nature of the action current in the frog's sciatic nerve we have practically invariably found it possible, by carefully reducing the strength of the stimulus, to eliminate the subsidiary waves and leave an alpha process which presumably still is maximal. We have not made accurate determinations in terms of some unit, of the range of strength of stimulation within which these changes occur, and, therefore, for the present can only say very generally, fully cognizant of the vagueness of the statement,

Fig. 7 (expt. 6, 12, '23). Two contact prints of the action current in the bull frog's sciatic nerve superimposed to show the shift in position, and the absence of any change in form, of the alpha process of the action current with an increase in the strength of the stimulus from just maximal for alpha to strongly supramaximal. These action currents are built up on the logarithmic disturbance, and their starts (marked) have been located by comparison with a record of the logarithmic disturbances alone (not shown here). X = 6.9 cm.; 1 mf.; 1,000$^{\omega}$. The lines extending down from the base line indicate successive 0.5 σ. Natural size.

that between the completely developed action current and an action current consisting apparently of a maximal alpha wave alone, there may be a range of stimulation strength that is determined by a shift of the secondary coil through a distance of 1 cm.

Increasing the strength of the stimulus from one just maximal, for the whole, or for the alpha process to one that is supramaximal causes a very definite forward shift in the position of the action current on the fluorescent screen. When the lead is far enough away from the stimulus to be out of range of the escape of the shock, the action current or its alpha process, as the case may be, shows no, or at least no definite, change in form. This is illustrated by figure 7 (expt. 6, 12, '23). Here the for-

ward shift with the increase in the strength of the stimulus from one just maximal for alpha to one strongly supramaximal for the whole process amounts to 0.18σ as measured between the starts, and 0.22σ as measured between the crests. As the crest separation can be measured much more sharply than the start separation, the former may be taken as a measure of the shift; this difference between the two measurements, though, is well within the limit of error and consequently is regarded as of no significance. On account of the long conducting distance of 10.4 cm. here obtaining beta has lagged practically completely behind the crest of alpha; therefore, the entrance of beta into the make-up of the action current elicited by the stronger stimulus cannot be a factor in the shift of alpha. That this is the case is proved by the fact that alpha increases little if at all in height when beta appears. The action current was traveling in the case of the experiment illustrated by figure 7 at the rate of 44.1 M.p.s.; if spread of stimulus were the only factor concerned, the shift in this case would be accounted for by an increase in spread amounting to 9.7 mm. This might very well account for the whole of the shift, and there are some reasons for believing that it is the most important factor. Further experiments are, however, being done in an effort to ascertain and evaluate the factors concerned in producing this shift with the increase in the strength of the stimulus. The fact of the shift is mentioned here merely because it has been necessary to take it into consideration in the interpretation of some of the records illustrating subsequent sections of this paper.

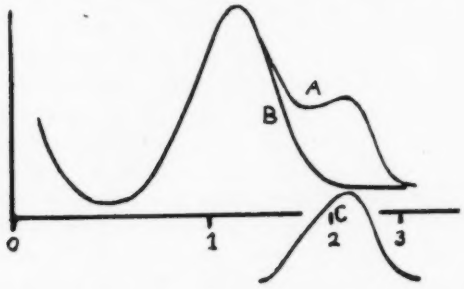

Fig. 8 (expt. 6, 2, '23). Tracings, *A*, of an action current (A of fig. 9) produced by a maximal stimulus for both alpha and beta, and, *B*, of a stimulus maximal for alpha but subminimal for beta (B of fig. 9) superimposed and subtracted, with *C* as the remainder. The lines extending down from the base line indicate successive σ. Natural size.

Analysis of the action current by graded stimulation. When the waves disappear in succession as the strength of the stimulus is gradually and steadily diminished the total duration of the action current decreases in steps. That this step-like shortening is due to the disappearance of definite waves in succession can be proved by obtaining the difference between appropriate records. Figure 8 (expt. 6, 2, '23) illustrates a typical result of such a subtraction. *A* is a tracing of the action current

the original of which is reproduced as *A* in figure 9; it is composed of an alpha and a well developed beta wave. *B* is a tracing of the same action current obtained when the stimulus was weakened until the beta process had disappeared entirely from view (see *B* of fig. 9). As the alpha process suffers practically no diminution in height it may be concluded that the stimulus still is maximal for alpha. The ascending limbs of the alpha waves of these two figures are here superimposed (disregarding a slight and insignificant shift necessitated by the effect of the difference in the strength of the two stimuli) and the difference between them is determined. The result is the curve, *C*, which, there are good reasons for believing, is practically a pure beta process. Here, therefore, have been provided the means of comparing the configuration of alpha with that of beta. In this experiment in determinations with stimuli of three different strengths the time to maximum of alpha varied between 0.67 and 0.83σ. The time to maximum of beta as determined by subtraction is 0.67σ. Because of the difficulty of deciding just where *A* and *B* begin to diverge from each other it is possible that the time to maximum of beta has been underestimated. The total duration of these processes is for alpha 1.83, and for beta 1.71σ. These waves therefore are essentially alike in duration and in time to maximum.

If the absence of waves in the action current as obtained at the shorter conducting distances is due to a more complete fusion, it should be possible under the right conditions to obtain from it by cautious reduction of the stimulus an action current differing from the former by the absence of beta (and of gamma). That this is feasible is demonstrated by the tracings included in figure 4. At the long conducting distance (76 mm.) this action current is composed of an alpha and a beta process (gamma very indistinct); while the action current obtained at the short conducting distance of 15 mm., *E*, is almost simple in form. By reducing the strength of the stimulus to a point where there seemed to be a temporary interruption in the reduction in the height of the record an action current was obtained of the form designated α. Upon subtracting the second of these waves from the first the dotted wave labeled β results. That these, α and β, actually compose the action current as obtained at the distance of 76 mm. is indicated by placing β at a distance from α equal to the lag that would develop if both started from the same point and traveled a distance of 76 mm. each at its determined propagation rate, and then adding the waves (see *D*, fig. 4). The result, it is seen, is a curve (*C*, fig. 4) that fairly closely approximates in form the action current actually obtained at that distance of conduction (76 mm.).

Here again it turns out that the two waves, alpha and beta, have approximately the same durations. Indeed, it is seen in figure 4, and to this attention already has been called, that the line *m-n* determined by

the projections of the crests of the beta waves upon the base lines intersects the zero base line practically at the projection of the crest of the dissociated alpha wave upon it. It may be stated here that the time relations of the alpha and beta waves thus dissociated are the ones that were employed for the construction of the diagrams shown in figure 5.

The fact that the dissociated beta wave has the configuration of an action current constitutes presumptive evidence that it is an action current; and the fact that it, as well as the additional waves, have their own thresholds of stimulation indicates that it, as well as the additional waves, are action currents in discrete sets of fibers, and, finally, the fact that the line joining the projected crests of the betas of the action currents obtained at different conducting lengths as plotted in figures 3 and 4 also intersects approximately the projected crest of alpha, practically proves that the alpha and beta processes start simultaneously under the stimulus. Though the gamma process cannot, on account of the smallness of its size, be followed as satisfactorily as the beta process, there nevertheless is every reason for believing that it, and possibly also the delta process, is similar to the alpha and beta processes in these respects.

The refractory phases of the alpha and beta processes and the analysis of the action current by the refractory phase method. If any further evidence is needed in support of the view that these waves represent sets of discrete action currents traveling in distinct groups of nerve fibers, it is furnished by the fact that the waves, or better the structures in which they arise, have different absolute refractory phases. It is proposed to deal specially with the subject of the refractory phase in a separate paper where methods and detailed results will be presented. Here only those aspects of the subject will be touched on that prove the view expressed above with regard to the nature of the waves.

The absolute refractory phase may be defined as the time that must elapse after delivering an effective stimulus, or after the arrival of an action current at a given point, before another response to a second stimulus is obtainable from the same point. It is not difficult by our methods to ascertain with considerable accuracy the refractory phases of the alpha and beta or even of the gamma, processes. By these methods it has been found that they are distinctive for each wave. To illustrate by a single typical example, the refractory periods of alpha, beta and gamma in one case (expt. 3, 30, '23) were 1.42, 2.06 and 4.46σ respectively.

Of even greater present interest, however, is the experimental dissociation of the action current into the several processes of which it is composed, that is made possible through the fact that the alpha and beta (and gamma) processes have different stimulation thresholds as well as different refractory phases. If by properly selecting the strength of stimulation, an action current consisting solely of a maximal alpha proc-

ess is started in a nerve, and if while the nerve still is absolutely refractory to this alpha process it is stimulated a second time through the same electrodes with a strong shock, one that ordinarily would elicit all of the processes, the action current started by the second stimulus will be without an alpha process.

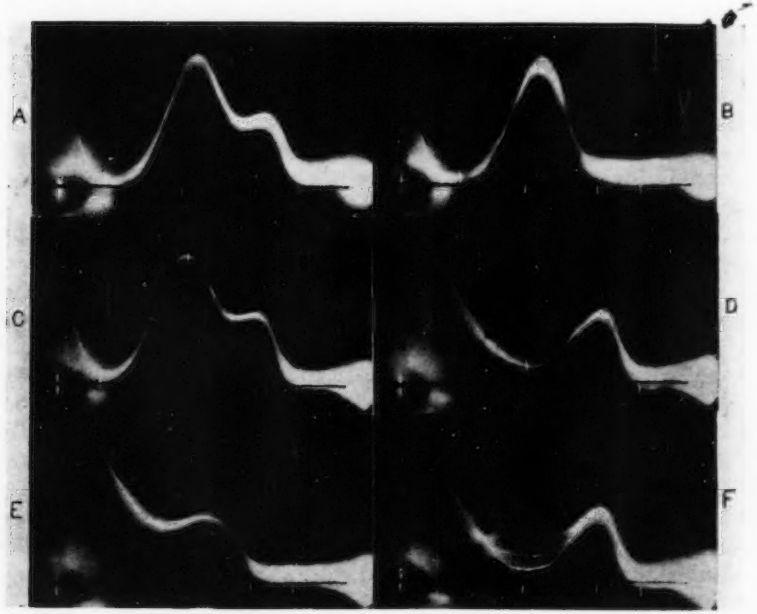

Fig. 9 (expt. 6, 2, '23). Records of the action current in the bull frog's sciatic nerve illustrating the isolation of the beta process by stimulating first with an induction shock that is maximal for alpha, but subminimal for beta and then, after an interval short of the absolute refractory phase, with a stimulus that is maximal for both. A = first action current alone, stimulus strong; B = first action current alone stimulus below the threshold of beta and gamma; C = second action current alone, stimulus strong; D = C following B (carried forward) by 0.85 σ; E = A carried forward as while making D; F = same as D but with unsteady first stimulus; X = 7.6 cm.; 1 mf.; 2,000$^{\omega}$. The lines extending below the base line indicate successive σ. $\times \frac{11}{17}$.

The steps of such an experiment are illustrated by figure 9 which is composed entirely of reproductions of direct contact prints. The action currents in this figure have all traveled the full length of the nerve, 82 mm. A record was first made of the action current produced by the first stimulus when strong (*A* fig. 9). It exhibits distinct alpha and beta waves and a very faint gamma wave. Then the stimulus was reduced until the beta process had completely disappeared; the record *B*, gives no evi-

dence of a beta process; the action current is simple in form. As there is no appreciable diminution in the amplitude of alpha the stimulus undoubtedly still was maximal for the alpha process. Next, the stimulus which produced the latter record was succeeded through the same stimulating electrodes by a second and stronger stimulus made by trial to follow the first by a time interval short of the full absolute refractory period of the alpha process. The interval actually used (determined subsequently by calculation) was 0.85σ. The action current resulting from the second strong stimulus alone is shown as *C;* it is identically the same in form as *A*. The tracing obtained by preceding this with the weaker stimulus that produces the alpha process alone, *B*, is shown as *D*. This record is made up of the end of alpha of the first action current, *B*, moved forward so that only the declining end now appears in the record, and of the isolated beta of the second action current. It might be suspected that instead it consists of the end of alpha and the whole of beta of one and the same (the first) action current. That this is not the case can be made clear in a number of ways. *a*, It may be repeated that the action current produced by the first stimulus was without a beta process, *B*. *b*, When the nerve is stimulated with only the first induction shock but strengthened to the point where beta appears (as in *A*) and the resulting action current is recorded in the advanced position it occupied while making record *D*, its beta wave (record *E*) occupies a much more advanced position in the record than that of the wave in *D*. *c*, In another record, *F*, made as was *D*, a flickering and faint deflection appears (reinforced for reproduction) in the position where either beta of the first action current or alpha of the second might well belong. It undoubtedly is the result of an irregular first stimulus which occasionally either rises above the threshold of beta, and beta of the first action current therefore appears, or falls below maximal for alpha of the first action current and alpha of the second therefore occasionally appears. For reasons which do not concern us here the latter of the alternative explanations is accepted as the more probable. But in any event the record proves by the position this unsteady deflection occupies, that the wave in *D* is not beta of the first action current. *d*, When the starts and the base lines of records *C* and *D* (the same action current but *C*, with, and *D*, without, the α process) are superimposed the crests and ends of the beta waves are superimposable within the limit of error of the method, the discrepancy amounting to only 0.06σ. *e*, The crest of beta in *D*, where it is isolated, is later than the crest of beta in *A*, which shows the position of beta of the first stimulus when strong, by 0.95σ which is very nearly the time difference between the two stimuli (0.85σ). *f*, Finally, when the first stimulus is increased in strength, so that the resulting action current contains the beta as well as the alpha process, and is followed after the

same interval as was used previously (that is, while the wave is refractory not alone to alpha but also to beta) by a strong second stimulus, the wave seen in record *D* also disappears.

The wave in record *D*, therefore, is composed almost exclusively of the beta process. Its rising phase, unfortunately deformed somewhat by an irregularity in the undeflected base line, lasts about 0.91σ, its declining phase between 0.96 and 1.20σ, as compared with 0.83σ and 1.03σ, the durations of the rising and declining phases of the alpha process produced by the weak stimulus (record *B*), and with 0.67 and 0.71σ the durations of the rising phases of the alphas of records *C* and *A* respectively, compound action currents produced by strong stimulation. As thus determined, therefore, the phases of beta seem to be somewhat slower than those of alpha; the method cannot, however, be regarded as ideal for the determination of small time differences.

It should be recalled here that it was the beta wave of this action current that was derived by subtraction in figure 8. It is not without significance that the beta waves obtained by these two totally different procedures are, within the rather wide limit of error of the methods, identical in form.

The propagation rate of this separated beta process, directly determined, was 26.3 M.p.s. In two indirect determinations of the propagation rate of beta by the method which utilizes the lag of the crest of beta behind that of alpha, rates of 25.9 and 26.3 M.p.s. were found. This remarkable agreement is of interest for two reasons. In the first place it justifies the use of the time between the crests of the waves for the indirect determination of propagation rates of the component waves; and secondly, it confirms the finding recorded above that the times to maximum of the alpha and beta processes are essentially alike.

Significance of the amplitude of the waves. If the point of view adopted in connection with the discussion of the summation of potential waves in nerve is justifiable it would follow that the recorded potential of these waves is not necessarily indicative either of the real or of the relative potentials in the fibers producing them. Thus, though the amplitude of beta, for example, is less than that of alpha, it is conceivable that the difference is attributable rather to the the relative number of fibers concerned with these two processes than to any difference in the potentials developed in association with the action current in the fibers active.

Length of the waves. All of the evidence we have succeeded in gathering indicates that the alpha and beta waves have approximately the same duration and time to maximum; occasionally beta has been slightly longer than alpha. Whether the same is true of the gamma and delta processes also it has not been possible to decide, but they certainly are no briefer than alpha. Confining ourselves to alpha and beta only, it

follows from the expression $l = d\ V$ in which l is the length of a wave, d, its duration and V its velocity, since d is approximately the same for both alpha and beta, that the lengths of the waves of a given action current are directly as their propagation rates. To take round median values, the propagation rates of alpha and beta in the bull frog's sciatic at room temperature may be put at 42 and 25 M.p.s. respectively; and the time to maximum and duration at the longest conducting distance may be put at 0.7 and 1.8σ respectively. For these values the length of the rising phase and the total length of alpha are 2.94 and 7.56 cm. and of beta 1.75 and 4.50 cm. On the same basis the values for gamma, which has a propagation rate of about 15 M.p.s. would be 1.05 and 2.70 cm. These figures give values comparable with one another. The absolute values would be less, as will be made clear later on, if data for the duration were taken from leads closer to the stimulus.

The "diphasic" action current. A few records of the action current in this nerve have been made through leads both resting on uninjured nerve (so-called diphasic leads). They are of interest solely on account of the confirmation they give of the waves seen in the action current recorded through leads, one on the intact side of the nerve, the other on its killed end (so-called monophasic leads). A record of a "diphasic" action current picked up 53 mm. from the stimulus and with 20 mm. between leads is shown in figure 10. This may be compared with figure 11 which is a mathematical construction of a "diphasic" action current from a "monophasic" action current, the 76 mm. action current pictured in figure 3, composed of typical alpha, beta and gamma waves. The similarity of the record to the construction, when the difference in conducted distance is taken into account, speaks for itself. When the stimulus is weakened to the point where in the "monophasic" lead the beta and gamma processes disappear, the "diphasic" lead gives a *diphasic* action current of the form familiar to physiologists.

FORM OF THE ACTION CURRENT IN OTHER NERVES. The sciatic nerve of the bull frog and the phrenic nerve of the dog on account of their length are especially adapted to the purposes of this research. These nerves, therefore, have received our main attention. Some observations have been made, however, on a number of other nerves. In the light of the information secured from the more suitable nerves the interpretation of these additional observations in general becomes a relatively simple matter, and may, therefore, be presented in abbreviated form.

Sciatic nerve of the green frog (Rana pipiens). A set of replotted action currents from the sciatic nerve of the green frog is shown in figure 12. At the conducting distance of 30 mm. and better at the longest conducting distance obtainable, 42 mm., the uncovering of a beta wave and possibly of a gamma wave can be seen. The propagation rate of alpha here,

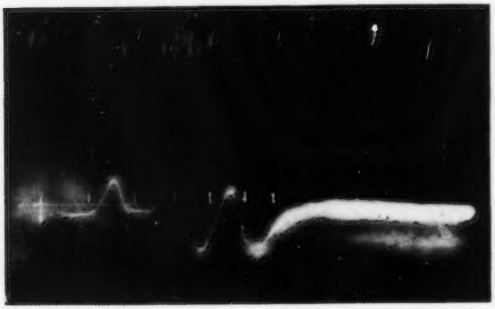

Fig. 10 (expt. 4, 17, '23). A "diphasic" action current in the bull frog's sciatic nerve. Conducted distance 53 mm. Distance between leads 20 mm. The first small wave is the "escape." X = 7.7 cm.; 1 mf.; 10,000$^{\omega}$. The lines on the base line indicate successive σ. Natural size.

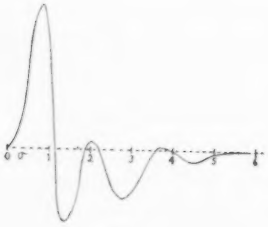

Fig 11

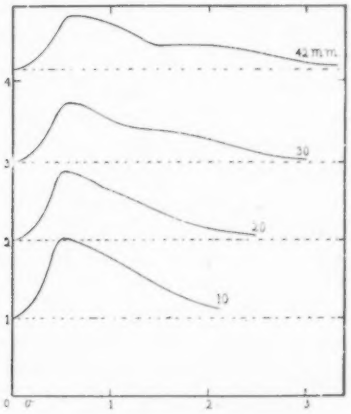

Fig. 12

Fig. 11. A "diphasic" action current constructed from a "monophasic" action current in the bull frog's sciatic nerve, that of figure 3, 76, upon the basis of a time separation of 0.5σ (corresponding with a conducting distance of 20 mm.) between the leads.

Fig. 12 (expt. 11, 22, '22). Tracings of the action current in the sciatic nerve of the green frog, transferred to linear coördinates, at four conducted distances, 10, 20, 30 and 42 mm. Temp. = 23.3°C. In the original, X = 6.15 cm.; 1 mf.; 3,000$^{\omega}$. Otherwise as in figure 3.

directly determined, was 33.3 M.p.s. (23.3°C.) and the calculated propagation rate of beta, 17 M.p.s.; the probable error, though, of the determination of the latter rate is large owing to the very incomplete separation of the waves at these conducting distances. These are usual values. Additional data will be found in table 1. Owing to the incompleteness of the separation of the waves it is not possible to ascertain whether the projected crests of the beta waves in figure 12 fall upon a straight line as they do in the case of the bull frog's action current diagrammed in the same way; nor is it possible to satisfactorily analyze this action current into its components by the method of subtraction.

Comparison of results obtained from the green and bull frog's sciatic nerves seems to show (see table 1) that though they both are similarly compounded of waves, in the former the time to maximum is shorter and the propagation rate is slower. The former also develops a higher potential amplitude. When, however, the comparison is made between the results obtained at comparable conducting distances, the differences are not so great as they at first sight seem. Thus while the time to maximum at the longest obtainable conducting distance in the green frog preparation (about 4 cm.) is about 0.5 to 0.6σ and in the bull frog (about 7 to 10 cm.) 0.7 to 0.8σ, in the latter at the conducting distance of 4 to 5 cm. values of 0.5 to 0.6σ are not infrequently obtained. And with regard to the recorded potential amplitude, this is influenced so much by the distance traveled and also by the relative amounts of active and inactive tissue in the nerve that the differences in observed potential possibly are without significance.

WARM-BLOODED NERVE. The phrenic is the only warm-blooded nerve that has been studied with any degree of completeness, though a sufficient number of observations have been made on other nerves to justify certain statements with regard to the configuration and propagation rates of their action currents. In one case the animal (dog) was kept under paraldehyde anesthesia and nerves were removed one after another for observation. The results obtained with mammalian nerve will be illustrated very largely by the data derived from this case, which may be regarded as typical of the limited number of observations we have made. During each experiment of this type the temperature of the moist chamber was maintained at a constant level, usually at about 38°C., and oxygen, saturated with water vapor, was added to its atmosphere.

The phrenic nerve. The data showing that the action current in the phrenic nerve is simple in form have already been presented (see fig. 1a). Since the separation of the compound action current into its components depends in large part upon the distance the action current is conducted, and since the longest preparations we have succeeded in making have consisted of the phrenic nerve of the dog, some of which have measured

11 cm., it follows, if the action current of the phrenic nerve is compounded in the same manner as is that of the frog's sciatic nerve, either that the potentials of its components are so low that they produce imperceptible waves, or that the propagation rates of such components are so nearly alike that they do not separate out even after traveling 11 cm. Neither of these alternatives seems acceptable.

Though waves do not appear in the phrenic action current, it does, as has been seen, change its form with propagation. This change in form, as has been pointed out, is similar to the change occurring in each of the waves of the compound action current in the frog's sciatic nerve; and amongst the possible ways of accounting for it one that has been suggested, is slight differences in the propagation rate of the action currents in individual fibers composing the nerve. Some conception of the range of rates that could produce this picture in the phrenic nerve upon this assumption is gained through a calculation based on the data of experiment 5, 16, '23 (see table 1). The propagation rate of the action current in this case being 73.5 M.p.s. and knowing the lag of the crest ($0.66 - 0.42 = 0.24\sigma$) developing during propagation through a known distance ($10.9 - 1.35 = 9.55$ cm.) it follows from formula 1 that if the component waves had the form of isosceles triangles the average rate of all of the action currents supposedly concerned in producing the phrenic action current in this case would be about 62 M.p.s. Further work on this subject is under way.

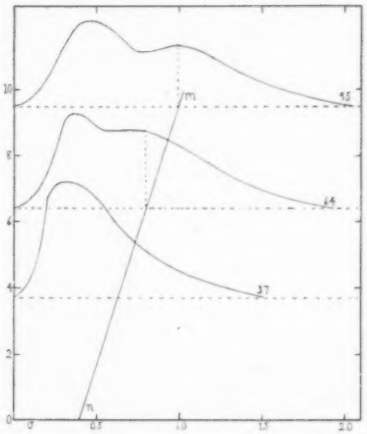

Fig. 13 (expt. 12, 16, '22). Tracings of the action current (A′ of fig. 14) in the saphenous nerve of the dog, transferred to linear coördinates, at three conducted distances, 37, 64 and 95 mm. Temp. = 38°C. In the original, X = 7.98 cm.; 1 mf.; 3,000ω; full potential. Otherwise as in figure 3.

Saphenous nerve. Figure 13 shows the action current in this nerve at conducted distances of 37, 64 and 95 mm., transposed to rectangular, linear coördinates in exactly the same manner as was done in the case of the bull frog's and green frog's sciatic nerve in figures 2b, 3 and 12. Obviously this action current is made up of two waves, alpha and beta, which separate out as the distance of conduction increases. Indeed, excepting the time relations, which are briefer, and excepting the absence of any indication of a gamma wave, this figure resembles in every respect

the comparable figures based upon records obtained from the sciatic nerve of cold-blooded animals, including not only the presence of waves, but also the narrowing of the crest as beta separates out, and the subsequent broadening of the crest, and the reduction in amplitude with propagation. Whether or not gamma is regularly absent from the action current of this nerve we are not prepared to say. The propagation rates of alpha and beta here are 83.4 and 60.7 M.p.s. This, it should be added, is the only instance in our experience with warm-blooded nerve in which the crest of beta has separated out sufficiently to make possible even an approximate determination of its propagation rate. It will be noted that though the conducting distance in this case (95 mm.) is longer than in the case of the bull frog's sciatic illustrated by figure 2b, where it was 82 mm., the separation of beta from alpha is considerably less complete. This is due to the closer approximation of the propagation rate of beta to that of alpha (ratio = 0.73) in this nerve than in the bull frog's nerve (ratio 0.6).

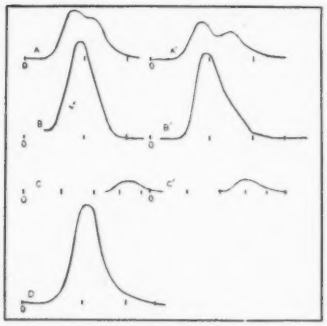

Fig. 14 (expt. 12, 16, '22). Tracings of the action currents in nerves removed in succession from one dog. *A* and *A'*, left and right saphenous at 77 and 95 mm. (3,000$^{\omega}$); *B* and *B'*, left and right tibials at 55 and 82 mm. (3,000$^{\omega}$); *C* and *C'*, left and right vagi at 87 and 80 mm. (5,000$^{\omega}$): *D*, left phrenic at 110 mm. (3,000$^{\omega}$). The marks indicate successive σ in each case. Very slightly reduced.

In the *tibial nerve* the action current gives evidence of alpha, beta and gamma waves (fig. 14). The waves, however, are so indistinct that a determination of lags and consequently of the propagation rates of beta and gamma is impossible. The indistinctness of the waves here is due not only to the relatively slight differences between their propagation rates, but to the shortness of the preparation in addition.

The only other mammalian nerve we have studied with any degree of care is *the vago-sympathetic trunk*. Though a number of attempts have been made to record the action current in this nerve, the very best result we have succeeded in obtaining is the one pictured in figure 14. We have no explanation to offer of the minuteness of their potentials. It is possible that our stimuli were inadequate, though with regard to this we can only say that the stimuli employed sufficed to inhibit the heart. Even if this action current were composed of waves it would be impossible to detect them in such minute deflections. The conduction rate in this nerve in two determinations was 53.6 and 58.7 M.p.s.

We have made only one observation on the action current in the *greater splanchnic* nerve. A tracing of the record obtained is shown in figure 15. The propagation rate was 9.6 M.p.s.

Figure 14 is composed of tracings of the action currents obtained at the longest conducting distances available in each case from nerves removed in succession from one and the same dog. The figure illustrates the similarity between the action currents in nerves of corresponding pairs, or conversely, a certain individuality of the action current in any given pair of nerves when allowance is made for the influence of conducting distance upon the form of the action current.

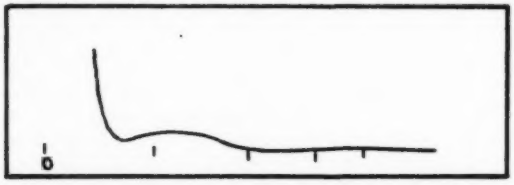

Fig. 15 (expt. 3, 7, '23). Tracing of the action current in the greater splanchnic nerve of the dog. Conducting distance 23 mm. The action current starts out of the descending phase of the escape. Temp. = 37°C.; X = 6.58 cm.; 1 mf.; 15,000$^{\omega}$; full potential. The marks indicate successive 5σ. Natural size.

THE ACTION CURRENT IN CLOSE PROXIMITY TO THE ELECTRICAL STIMULUS. We present next the method of observing the action current close to the site of its origin and some of the results obtained through its use. Action currents close to the site of stimulation are greatly distorted by the escape of the stimulating current; consequently for the interpretation of the records it is essential that the escape be not too large and that a record of it alone be obtained for subtraction from the combined deflection. This summation of escape and action current differs in nature, presumably, from the summation of the waves of the compound action current, in that the escape establishes a difference of potential between the grid and the ground of the amplifier which is in effect a changing grid bias. The potential of the action current is then recorded on this bias as a base line.

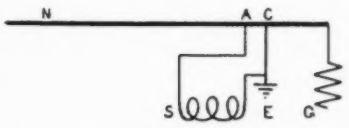

Fig. 16. Diagram of the connections for the registration of the action current under the stimulating electrode. N = nerve; A = anode and C = cathode of the secondary circuit, S; G = the grid and E the ground connections through non-polarizable electrodes on the nerve.

The best preparation for the purposes of these experiments is the sciatic nerve of the green frog, because the potential of its action current is high and of its escape relatively low, the latter possibly because the

irritability of the preparation is high; in other words, the phenomenon under investigation is prominent and the disturbing factor relatively insignificant. In one instance in a green frog nerve a legible record of the action current has been obtained where it develops at the point stimulated; figure 16 shows the arrangement of the connections in this experiment. In the case of the bull frog's sciatic nerve, legible records could not be obtained at a separation of stimulus and lead of less than 10 mm.; of the dog's phrenic, at a separation of less than 13.5 mm.

Three methods in general have been employed for the purpose of ascertaining the form of the escape. *a*, When the lead is directly from the stimulating cathode, as in figure 16, or close to it, the form of the escape by itself may be obtained, after having made the combined record, by placing another stimulus earlier by such an interval that the stimulus first used now falls in the absolute refractory phase of a preceding action current; or *b*, after having recorded the action current, the nerve may be anesthetized and the escape then recorded. *c*, Where the cathode and lead have a space between them, after having first recorded the action current, a short length of the nerve under the lead may be killed and the escape then recorded. None of these methods yields entirely unambiguous results. Thus the escape alone sometimes rises higher and more steeply than the escape combined with the action current. If the form and amplitude of the escape depend in part upon the electrical capacity of the nerve this difference might well be due to a diminished capacity possibly connected with the changed condition of the nerve. However this may be, on account of such changes in the form of the escape it sometimes has been necessary in order to make the records fit together, to arbitrarily reduce the record of the escape vertically (see fig. 2b). Then, too, at the very high rate of horizontal movement of the recording spot required in these experiments sometimes 70 M.p.s. at the end of the first centimeter and 33 M.p.s. at the end of the second, a time shift amounting to as little as 0.01σ in the positions of the curves to be compared, may very decidedly affect their superimposability. Nevertheless the short lead records obtained by these methods have given information that is of some interest if it is not entirely unequivocal. It is hoped that there will be opportunity later for a more thoroughgoing study of this phase of the subject. Pending further observations the experiments will be utilized only for the purpose of obtaining the form of the action current at, and close to its initiation.

Two records made by these methods have already been depicted and referred to, one from the bull frog's sciatic with a conducting distance of 12 mm. (see fig. 2b) and one from the dog's phrenic (figs. 1, a and b), with a separation of 13.5 mm. Figure 17 portrays the analysis of the set of records in which the lead was directly from the stimulating cathode in

the green frog's nerve. *C* is the action current combined with the escape. *B* is the escape. It was obtained by putting the stimulus in the absolute refractory phase of a preceding action current. It is not, however, a pure escape since the preceding action current runs over into the combined record. A record of this part of the preceding action current by itself was therefore made and transferred to this figure, where it is seen as *D*. To get the actual form of the escape, *D* is subtracted from *B*, giving *N*, the escape. Then by subtracting *N* from *C*, the action current, *A*, emerges. The perfectness of the curve formed by the points found by these subtractions gives some assurance that the actual form of the action current has been derived. The very beginning of the action current here is not determinable because of the steepness and faintness of the records in that position. It can, however, be definitely stated that the interval between the rise of the action current potential and the beginning of the escape potential, *B*, is not over 0.06σ in duration.

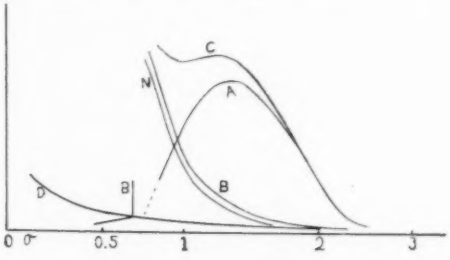

Fig. 17 (expt. 2, 17, '23). Derivation of the action current from its combination with the escape when the lead and stimulating electrode are one. Sciatic nerve of the green frog. For the subtraction an enlarged (2.72×) photograph was made through millimeter coördinates on glass. Description in text. Temp. = 22°C.; X = 7.7 cm.; 1 mf.; 3,000ω; shunted by 11,875ω. Natural size.

Additional data from this experiment and relevant data from the remainder of the experiments of this type are collected in table 2. There it can be seen that the time to maximum of the action current is short, ranging between 0.41 and 0.55 (in one instance 0.61), as compared with a range between 0.51 and 0.81σ in comparable experiments in which the conducted distance was long. The total duration also is short, 1.14 to 1.75σ (in one experiment 2.05σ), whereas after conduction the length of the nerve the duration of the action current usually exceeds 3.0σ. A large part of this increase, though, is due to the separation out of the slower waves. A fairer comparison is with the pictures of alpha and beta isolated either by subtraction or by the refractory phase method after propagation down the nerve. In such the duration of alpha and beta, it will be recalled, are approximately alike and range between 1.71 and 2.11σ. These results merely serve to supplement the observation that the duration and time to maximum of the action current waves increase with the distance the action current is propagated.

TABLE 2

Data of close lead experiments

EXPERIMENT	PREPARATION	CONDUCTED DISTANCE	STRENGTH OF STIMULUS	TIME ESCAPE TO START*	TIME TO MAXIMUM		DURATION
					In close lead	In distant lead†	
		mm.		σ	σ	σ	σ
2/17/23	Green frog sciatic	0	Just supramaximal	0.06	0.55–0.61	–	1.75
6/17/23	Green frog sciatic	1	Supramaximal	0.07–0.12	0.46–0.49	–	2.05(?)
6/17/23	Green frog sciatic	3.5	Strong	0.11	0.41	–	1.40
5/18/23	Green frog sciatic	5.0	Weak	0.15+	0.43	0.51 (2.45)	1.40
5/30/23	Green frog sciatic	9.5	Threshold	0.38(?)	0.31(?) 0.33(?)	0.61(?) (3.1) 0.68 (3.1)	–? 1.36
6/12/23	Bull frog sciatic	10.0	Threshold Strong	0.19 0.05	0.41 0.50	0.81 (10.4) 0.77 (10.4)	(?) 1.14
5/23/23	Bull frog sciatic	12.0	Slightly supramaximal	0.20	0.48	0.80 (8.2)	1.60
5/16/23	Dog phrenic	13.5	Supramaximal	?	0.42(?)	0.66 (10.9)	1.02(?)

* These values are influenced not alone by conduction, but also by the utilization period and by the spread of the stimulus. Suitable data for the evaluation of these factors are not at present available.

† The figures in parentheses give the conducting distance in centimeters.

The duration of the compound action current close to the site of its origin must be that of its longest component; I therefore neither alpha, nor beta, nor gamma here ordinarily ends in less than 1.40 to 1.50σ. Increasing the strength of stimulation through a range, which at a distant lead would give everything from a submaximal alpha process to a complete, waved action current, results in only a very slight, if any, increase in the duration of the action current when the lead instead is from the nerve at the site of stimulation. If there is any increase in duration it does not exceed 0.12σ.[6] This may be taken to indicate again that the beta and gamma processes are little if any longer in duration than the alpha process.

The time to maximum of the action current at the site of stimulation must be the mean time to maximum of all of the component processes. If we take 0.50 and 0.75σ as the times to maximum respectively of the action current at the site of origin and of the alpha process after conduction a distance of 8 cm. in the sciatic of the bull frog, and if we take 42 M.p.s. as the rate of propagation of the foot of this process, then if the prolongation of the wave during propagation is due to differences in the propagation rates in the constituent fibers and if the constituent action currents are assumed to have the form of isosceles triangles, it follows that the mean rate of propagation of the constituent action currents is about 37 M.p.s. or 5 M.p.s. slower than the fastest constituent of the action current.

Discussion and crucial experiment. Turning now to a consideration of the nature of the waves of the compound action current, the evidence we have thus far presented is so clearly in favor of their being discrete action currents originating simultaneously under the stimulating electrode and traveling along the nerve at different rates, that no other plausible way of accounting for them seems possible. In our preliminary statement (2), published before this evidence had become so convincing, other possibilities were mentioned, but even at that stage of the investigation these were easily disposed of. The waves cannot, for instance, be due to strong stimulation because they are not evident in the phrenic nerve under any circumstances. For the same reason they cannot, be attributed to the development of discrete, regular, decremental states resulting from injury to the nerve in preparation. As a matter of fact injuring the phrenic nerve by manipulating it roughly, or by painting it with scalding water, or with 95 per cent alcohol, does not cause waves to appear in its action current.

The phrenic differs from the sciatic nerve in that branches in the former are insignificant (4), but constitute a striking feature of the latter.

[6] Experiment in collaboration with G. H. Bishop.

The advisability of ascertaining whether the branches have anything to do with the waved nature of the action current in the sciatic nerve became apparent during the course of some experiments that have recently been performed.[7] In these experiments it has been found that when an inert conductor, such for example, as an idle non-polarizable electrode, rests upon the nerve anywhere *between* the leads the action current is deformed by a notch or by a wave in a manner to be described elsewhere. Not only is this deformation produced by a foreign conductor, but also by the stumps of the nerve's own branches. It has, however, been found that the waves with which the present paper concerns itself are not due to the presence of branches. The waves appear when there are no stumps between leads; indeed they have been obtained when the preparation has consisted only of the part of a very large bull frog's sciatic nerve that is entirely free of branches.

The fact that waves never appear in the phrenic nerve precludes the possibility of attributing them to a "repetitive" process started by a prolonged stimulating current, such as Forbes and Gregg have described (5), or to any other similar mechanism. This is precluded also by the fact that the induction shocks that have been employed have a duration that is less than one third of the measured refractory phase in cold-blooded nerve. Furthermore, on account of this refractory phase it is impossible to duplicate by repeated stimulation the pictures of the more complete summation waves that are obtained at the shorter conducting distances. And finally, as will be shown in a subsequent paper, when a process is set up early in the relative refractory phase of a preceding disturbance its velocity curve has an entirely different form from that of the beta or gamma waves.

Since the publication of our previous reports on this subject (1), (2) Broemser (6), by mathematical treatment of the record of the action current in the frog's sciatic obtained by connecting the side and dead end of the nerve through a string galvanometer, has derived a figure consisting of several oscillations around the base line. These oscillations he attributes to reflection of the action current at the injured end of the nerve. Brücke (7), however, has shown that reflection of waves from the cut ends of the nerve will not account for Broemser's derived pictures. As a matter of fact, the cathode ray oscillograph shows that the waves obtained through end to side leads are entirely monophasic and therefore cannot be attributed to reflection.

The foregoing considerations, together with the fact that the waves have different thresholds of stimulation and especially different refractory phases, leave little room for doubting that each of the waves is an action

[7] In collaboration with G. H. Bishop.

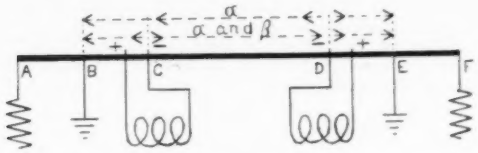

Fig. 18 (expt. 6, 26, '24). Diagram indicating the plan of the experiment proving that the alpha and beta waves travel in different fibers. Description in text.

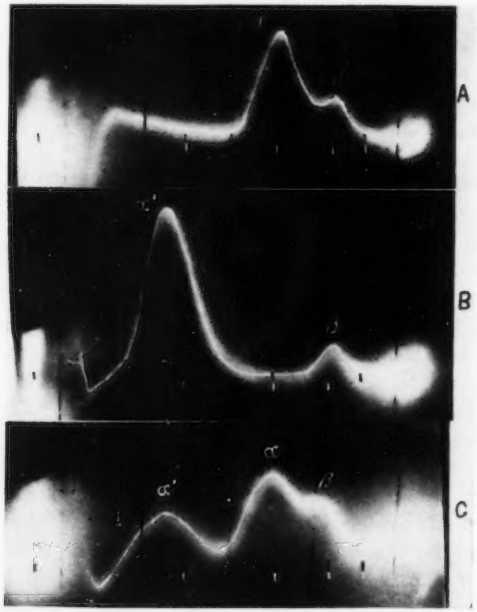

Fig. 19. Records of the action current in the bull frog's sciatic nerve obtained by the method shown in figure 18.

A = action current made up of both alpha (α) and beta (β) traveling from C to E (see fig. 18).

B = the same action current modified as a result of passing it through a maximum alpha process (α') going in the opposite direction. Only the beta process of the former remains.

C = same as B except that the alpha process (α') in this case is submaximal. Now in addition to the beta process some of the alpha process remains; the latter is reduced in height as compared with α in A. The black and white marks crossing the level of zero potential indicate successive σ. $\times \frac{14}{11}$.

current in nerve fibers having distinctive physiological characteristics. Quite recently[8] an experiment has been performed which proves beyond peradventure that the alpha and beta processes, and presumably also the gamma and delta processes, are propagated each in its own set of fibers. Figure 18 indicates the plan of this experiment. The sciatic nerve of a bull frog lies upon two pairs of leads, *A-B* and *E-F*, one at either end of the nerve, each pair connecting intact surface with dead end through the recording mechanism. Arrangements are made to stimulate the nerve with induction shocks at *C* and at *D* between the grounded leads, *B* and *E*, into the amplifier. To prepare for the main test a break shock is found which is just maximal for both the alpha and the beta processes. This stimulus applied through electrode *C* produces the action current *A*, figure 19, as recorded at *E* after propagation a distance of about 60 mm. Another break shock is found which is maximal for alpha but subminimal for beta. It is applied to the nerve through electrode *D* and the resulting action current is recorded at *B*. Then both of these stimuli are applied simultaneously while recording through *E*. The action current starting from *D* will travel not only toward *B*, but also toward *E*. At the latter electrode it appears as α' in figure 19, *B*. The two action currents started by the simultaneous stimuli will meet at a point midway between *C* and *D* (about 25 mm. from their origins) and to reach *E* the alpha and beta processes from *C* must pass through the alpha process from *D*. The record, *B*, figure 19, shows that the alpha processes mutually block each other; the alpha process fails to reach *E*. On the other hand the beta process, β, goes through without change in propagation rate or in form. This could happen only if the alpha and beta processes were traveling in different fibers. By means of a slight modification of the conditions in this experiment justification for the interpretation made above is readily obtained. If instead of a maximal, a submaximal alpha process is sent from *D* to *B* then, as shown in figure 19, C, this submaximal alpha process, α', only partially blocks, that is reduces height of, the alpha process, α, traveling in the opposite direction, while again the beta process, β, passes on unchanged.

The waves then represent discrete action currents in different nerve fibers. In the present state of our knowledge it is, however, impossible to assign to the waves definite functions; and speculation on this phase of the subject based on the results we have thus far accumulated has failed as yet to suggest even a satisfactory working hypothesis. To be sure it is not at all surprising in view of the varied histological composition of mixed nerves (8) to find that the action current of such a nerve as the sciatic is compound. Indeed the varied histological composition of nerve has

[8] In collaboration with G. H. Bishop.

led Langley (9) recently to venture the opinion "that the size" for instance "of the fibers has some definite connection with the kind of tissue in which the fiber ends." Now that the action current of a mixed nerve has been found to be compound one would naturally be inclined to infer that each component is concerned with the mediation of some particular type of response. For a clue to the solution of the problem one would naturally turn first to the two great groups of fibers of the peripheral nervous system, namely, motor and sensory. It is, however, currently believed that the conduction rate of the action current is of the same order of magnitude in afferent and efferent fibers (10). The fact that thus far we have observed only two waves in the action current of the saphenous nerve (dog) whereas the action current of the tibial nerve dissociates into three, sometimes into four, waves, would naturally lead one to attribute this difference to the absence of voluntary motor fibers in the former nerve. But this possibility seems to be precluded by our data in that they show that the leading wave both in the saphenous and in the sciatic action currents has essentially the same rate of conduction. That motor and sensory fibers of a mixed nerve have discrete action currents traveling at different rates is negatived also by the form of the action current in the phrenic nerve. Despite the fact that this nerve contains, in addition to motor fibers, "an abundance of fibers from sensory ganglia" (8) its action current has always been simple in form.

If the waves are action currents in motor fibers it might be that they are indicative of group innervation of groups of muscles. Ritter's observation, confirmed by Rollett (11), that upon stimulation of the sciatic nerve with electrical currents of gradually increasing intensity the muscles innervated by the peroneal branch first contract and later those innervated by the tibial branch, would seem to fit very nicely into the picture we obtain upon similarly stimulating this nerve: owing to differences in the thresholds of the waves composing the action current, alpha, it will be recalled, first appears as the current strength is increased, then beta, etc. It, however, remains to be determined whether the fibers composing the peroneal and tibial branches actually conduct the impulse at different rates.[9] But in any event it is obvious that the waves in the saphenous nerve cannot be accounted for on any such basis as this. The fact that the waves have different propagation rates suggests that they might be in groups of fibers belonging to different anatomical segments

[9] Recently, in collaboration with G. H. Bishop, it has been shown that the action current in the central end of the sciatic nerve of the bull frog resulting from stimulation of its peroneal and tibial branches separately have identically the same form and propagation rate, and that in both alpha and beta waves separate out. This is true also for peripheral conduction when the trunk is stimulated and leads are taken from the tibial and peroneal branches separately.

and going, therefore, to groups of muscles at different distances from the central nervous system. This cannot, however, be the case for if it were the general configuration of the compound action currents traveling the full length of the preparation in the afferent direction on one hand and in the efferent direction on the other would not be the same, and it is. Opposed to this view, furthermore, are the differences in the other physiological properties (the refractory phase and threshold stimulation) of the fiber-groups, since these could serve no obvious purpose on the basis of segmental distribution.

The literature contains a number of references to an apparent relation between the rate of propagation of the impulse in a nerve trunk and the inherent quickness of the peripheral structures innervated by it (12), (13), (14). And Lapicque and Legendre (15) have published data connecting the irritability of nerve with both morphological and physiological differences. These investigators have found, for example, that the nerves having the shortest chronaxie are those of the largest caliber which they find go to the faster muscles. It would be very natural to suppose, therefore, that the alpha and beta processes are the action currents in the fibers innervating such structures, for example, as white and red cross-striated muscle. The presence of waves in the action current of the sciatic nerve and their absence from the action current of the phrenic nerve would not be inconsistent with this view. This hypothesis would not, however, account for the waves in the action current of the saphenous nerve.

Insofar as concerns motor impulses there is every reason for believing that the relation which has been described as obtaining between the propagation rate of action current and inherent quickness of muscle innervated really is a matter of adequacy of stimuli. Just what this means in physical terms is not at present known. But it seems probable that an adequate stimulus as related to muscle would be a nerve action current that presents the potential configuration that is best adapted to the stimulation of a given muscle. A feature of the action current which may be of significance in this connection is the potential gradient of its rise. Now, though alpha travels much faster than beta, the relation obtaining between the propagation rates and the lengths of these waves is such that their times to maximum at a given point are usually very much alike. Taking the data, however, as a whole the rising phase of beta is perhaps slightly longer than that of alpha. And if we assume that the potential of beta is less than that of alpha then the rate of change of potential in beta is slower. On the other hand should it be found that the potential gradients of the waves are alike and the waves nevertheless are action currents in fibers mediating different types of impulses it might become necessary to correlate adequacy of stimuli with some

other less obvious of the physiological properties of nerve, such, for example, as find their expression in differences in propagation rate, in threshold of stimulation, and in duration of refractory phase.

Differences in the nature of nerve impulses on the afferent side have been invoked by Head and collaborators (16), (17), if we read these authors aright, in order to account for the regrouping of somatic impressions which they describe as occurring within the central nervous system. "Each end organ in the skin," they write, "is capable of reacting to the mass stimulation of the environment in a specific manner. . . . . When these *peculiar impulses* (italics, ours) reach the spinal cord, they are discharged into secondary systems, each of which is guarded by specific receptors. . . . . It is as if the gallery of a concert hall were fitted with a series of resonators, each of which was tuned to a certain note." The indications we have obtained of differences in the properties of groups of fibers composing a nerve supply an experimental basis for the "peculiar impulses" which Head and Thompson find it necessary to predicate in order to account for their clinical observations. Granting the existence of peculiar impulses in particular nerve fibers, the only further condition that would be needed to effect a regrouping of somatic impulses is that the secondary systems, in the sense of Head and Thompson, respond most readily, as do the peripheral receptors, to their adequate stimuli.

These few examples will suffice to indicate the possible applications of our findings that the fibers composing a mixed nerve are not all alike in a physiological sense. To repeat, these differences may mean that the stimuli the fibers deliver are different and perhaps adequate to the structures they innervate. It should be made clear, however, that we have not yet succeeded in obtaining any direct experimental evidence that can be regarded as proving the existence of differences in the stimulating values of the impulses delivered by the different types of fibers. The results of our investigations do, however, prove that in certain of the mixed nerves the action current is compound. Furthermore they indicate the possibility, which is being investigated further, that each of the potential waves of any action current is a composite of potential changes slightly out of phase in individual nerve fibers. However this may be, we suggest for the sake of convenience that action currents from which discrete waves separate out be called *compound* and that the potential waves which lengthen on propagation be designated *simple* action currents.

SUMMARY

The action current differs in form in different nerves and with the distance it is propagated from the stimulating induction shock.

In the phrenic nerve of warm-blooded animals its form is simple; as it progresses along the nerve there occurs a gradual though slight lengthening of all of its phases and a diminution in its amplitude.

In the sciatic nerve of the bull frog and of the green frog, and in the tibial nerve of the dog the action current under or close to the stimulus also is simple in form; but as it moves along the nerve it dissociates usually into three, sometimes into four waves. In the saphenous nerve of the dog the action current usually breaks up into two waves.

The waves are regarded as discrete action currents traveling in fibers possessing different physiological characteristics. They have approximately the same duration, but different propagation rates and therefore different wave lengths. The fiber groups have distinctive thresholds of stimulation and duration of refractory phase.

Each of the waves of these compound action currents as it progresses along the nerve changes its form just as does the simple action current in the phrenic nerve. It is suggested that these changes are due in part, at least, to slight differences in the propagation rate in individual, or in many small groups of fibers, whose action currents therefore get slightly out of phase as they progress.

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